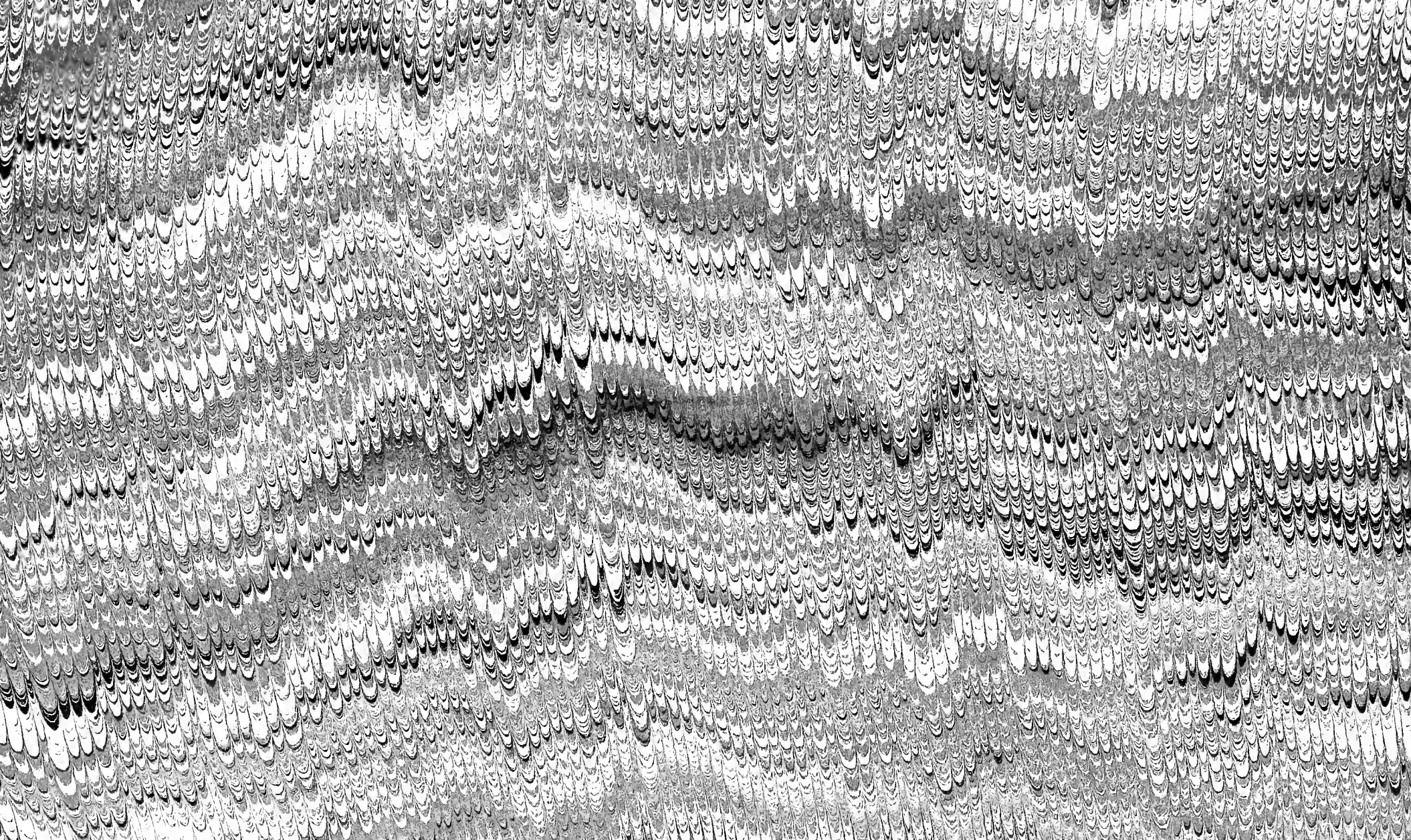

# PARASITOLOGY

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*CAMBRIDGE UNIVERSITY PRESS, FETTER LANE, LONDON*
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VOLUME VI OCTOBER, 1913 No. 3

# THE ANATOMY OF *ARGAS PERSICUS* (OKEN 1818).

## PART II.

BY L. E ROBINSON, A.R.C.Sc. LOND.,
AND J. DAVIDSON, M.Sc. LIVERPOOL.

(*From the Cooper Laboratory for Economic Research, Watford.*)

(With Plates XIV–XVII and 8 Text-figures.)

### TECHNIQUE.

THIS subject has already been considered in some of its aspects in the first part of this paper, but the methods which apply more especially to the study of the internal anatomy will now be dealt with.

For the study of the external forms and the relations of the larger organs, dissection with the aid of a dissecting-microscope, using magnifications of 10–20 diameters, is necessary. The knowledge derived from such dissections is a valuable aid to the interpretation of serial sections and is even, at times, indispensable.

Small shallow glass dishes, filled to a depth of three-eighths of an inch or so with paraffin coloured with lamp-black, are most serviceable for the purpose. For fixing the tick in position, a sufficient area of the wax is melted by means of a heated wire and, before it solidifies, the tick is placed in the melted wax and adjusted for dissection. If just removed from alcohol or other fluid, the specimen should first be carefully dried with a piece of filter-paper. After the wax has solidified, the tick may be more firmly fixed in position by light applications of the heated wire round the margins of the body.

Living ticks may be dissected under normal saline solution and the movements of the heart and the alimentary canal observed; or for the examination and removal of portions of the living organs etc. for sectioning. An objection to such dissection, and this applies also to the dissection of ticks which have been merely killed with chloroform, is the fact that in making the first incision through the cuticle, it is almost impossible to avoid wounding the more or less distended alimentary coeca, with the result that the ingested blood is constantly oozing and obscuring the dissection. If the tick is first killed by momentary immersion in boiling water, the contents of the gut are coagulated and the subsequent operations are thereby facilitated. This procedure, of course, makes it impossible to use the specimen so treated for the study of histological details, but, in our opinion, removal of organs or portions of organs, or partial dissection of the tick as a preliminary to histological fixation is undesirable. The handling of the tick induces strong contractions of the dorso-ventral body muscles and owing to the pressure thus created in the body cavity, extrusion and consequently displacement of the organs take place, immediately the pressure is released by the incision. Provided certain precautions are taken, it is not a difficult matter to ensure perfect fixation of the internal organs without further mutilation than is occasioned by the insertion of a hypodermic needle in the manner described below.

In addition to congealing the contents of the gut, the boiling water treatment leaves the organs, and tissues generally, in a condition suitable for dissection, which operation is carried out under water or normal saline solution. Alcohol makes the organs very friable and is therefore unsuitable, except for special purposes. Dissection from the dorsal surface is the most frequently used method, but it should be borne in mind that dissections from the lateral and ventral aspects are also of the greatest use. After the tick is fixed in the dissecting dish and covered with liquid, a small incision is made at the posterior margin of the body. The dorsal integument is then gently raised with fine-pointed forceps and the cuticle divided progressively, with a mounted needle ground to a cutting edge, along both lateral margins at the line of junction between the dorsal and ventral integument, where the cuticle parts readily. As the flap of skin is gradually reflected forwards, muscular and connective tissue attachments on its under surface are divided as they come into view.

Most of the errors of the earlier workers on the anatomy of ticks are the outcome of observations made from dissections alone, and, useful and

necessary as such dissections may be, they should always be checked and amplified by the study of serial sections of the entire tick, cut in the sagittal, transverse and horizontal planes, respectively.

For the fixation of ticks before section-cutting, we have found Carnoy's Glacial Acetic acid—Absolute alcohol—Chloroform Mixture, in the proportions 1:6:3 very satisfactory. In addition, Flemming's Fluid (strong formula) has given good results. For the demonstration of the secretion-granules in the salivary glands, Kopfsch's Mixture gives striking results, but is not a desirable reagent for general use. In addition to the foregoing, we have used Kleinenberg's Picro-sulphuric acid Mixture, Perenyi's Fluid and Sublimate-acetic (10% acetic acid saturated with $HgCl_2$). Reference to the use of the first mixture has been made in Part I of this paper (p. 24) and the other two reagents do not give satisfactory results. There is reason to believe that Gilson's Mixture—a modification of Carnoy's Fluid, with the addition of mercuric chloride—is an eminently suitable fixative, but the authors have had no personal experience of this.

As already pointed out, the thick chitinous cuticle of the tick is not readily permeated by fluids, and this makes fixation a less simple operation than usual. The mere immersion of the tick in the fixing fluid is not sufficient; and as mentioned above, incisions of the cuticle, made to assist penetration, lead to displacement of the organs. The most satisfactory method is to inject the fixative into the body by means of a hypodermic syringe. We used an "all-glass aseptic" syringe made by Messrs Burroughs Wellcome and Co., as being less subject to any corrosive action of the fixing fluids; the steel needle was protected by being dipped into hot melted vaseline and then drained, so as to leave a scarcely perceptible film of vaseline on the surface. The needles, as supplied, are too long in the orifice and it was found necessary to modify this defect by rubbing down the points to a more oblique angle. With care, a sharp cutting-edge may be maintained at the tip of the needle and no difficulty is experienced in penetrating the cuticle of the tick. The operation is carried out as follows:—the living tick is taken between the thumb and forefinger of the left hand, and the needle of the syringe, previously filled with the fixing reagent, is inserted *transversely* into the body, some little distance posterior to the anus, and as soon as the orifice in the needle is completely introduced, the fluid is slowly injected. In performing this operation, a sheet of glass should be interposed before the face of the operator to protect him from any accidental spurt of fixing fluid. As injection proceeds, the legs of the tick become stiffly

extended, in succession from behind, forwards, and finally the capitulum is depressed into the camerostome. When this occurs, the tick is immersed into a quantity of the fixing fluid in a small glass vessel, the needle is withdrawn, and the specimen remains therein for such time as is necessary to ensure complete fixation.

The specimens are then thoroughly washed with a suitable medium, and after dehydration embedded in paraffin. A short account of the procedure which we used is given in Part I (p. 24).

After fixation in Carnoy's Fluid, sections were stained in Thionin and counter-stained with Eosin or Orange G. If Flemming's Fluid had been used for fixation, Flemming's so-called "triple stain" (Safranin—Gentian violet—Orange G.) or Heidenhain's Iron-alum-Haematoxylin, was found most suitable. Materials fixed with Kleinenberg's Picro-sulphuric acid or Perenyi's Fluid were stained with Ehrlich's Haematoxylin and counter-stained with Eosin, Orange G. or Picric acid. As a contrast-stain, the latter reagent has the advantage of demonstrating the chitinous structures very clearly.

For the dissections, one of Zeiss's binocular, image-erecting dissecting microscopes was used. The microscopical examination of sections etc. was carried out with Zeiss apochromatic objectives and compensating oculars and all the figures were drawn with the aid of the Abbe drawing apparatus.

## The integument.

Plate XVII, fig. 1.

As already stated in Part I of this paper (p. 29), the integument consists of an external chitinous *cuticle*, and an internal *hypodermis* composed of living cells. The hypodermis of *Argas persicus* presents a very different appearance to that of the Ixodid ticks. With the exception of a limited area, it consists of a thin layer of cells with a finely granular protoplasm and flattened nuclei. Intercellular membranes are not apparent, and in consequence, the nuclei appear to be scattered at irregular intervals in a thin protoplasmic lining of the cuticle. The exception is the hypodermis which underlies the cuticle of the post-genital area, in which part of the body, only, do the hypodermal cells exhibit the columnar form which is the rule in the *Ixodidae*.

The hypodermal layer is invariably found accompanying the chitinous structures; even internal parts such as the invaginated portions of the cheliceral sheaths, the cheliceral shafts and the subcheliceral plate, all

have the hypodermal layer persisting on their coelomic surfaces, though in places it may be so thinned-out as to become almost unrecognisable.

A remarkable feature in *Argas* is the almost complete suppression of the *dermal glands*, which form such a conspicuous character of the hypodermis in the *Ixodidae*. The very few minute pores which are found sparsely scattered over both the dorsal and ventral surfaces of the body, are quite rudimentary, and the underlying hypodermis shows, at the most, a very vestigial gland-structure. In the case of *Ixodes ricinus*, Nordenskiöld[1] has observed that in old individuals, the originally columnar hypodermal cells become flattened, and the dermal glands disappear. We have noticed the same degenerative tendency in the hypodermis of other Ixodid ticks[2]. In the case of *Argas*, however, the description given above applies to the young adult forms, and to the nymph; concerning the hypodermis of the larva, we have no information.

It would appear, therefore, that in *Argas*, the hypodermis has fulfilled its main purpose when the cuticle is formed, and that it then assumes a quiescent state until the arrival of the next moulting period. The secretory function which is so well developed in the *Ixodidae* seems to be entirely suppressed.

As in other ticks, the cuticular layer shows an outer layer of dense highly-refractive chitin and an inner thicker layer of softer chitin which stains readily with acid dyes. The horizontal stratification which is so well shown in the deeper portions of the cuticle of the Ixodid ticks, is absent, but the delicate fibrillar *canaliculi*, which Nordenskiöld[3] has described and figured so beautifully in his work on *I. ricinus*, are very evident in suitably prepared sections of the cuticle. These canaliculi commence at the internal surface of the cuticle and run directly towards the external surface, some little distance below which they terminate blindly. Many follow a somewhat undulating course, but as far as can be seen, they do not branch or fuse with each other. We have not been able to assure ourselves that these cuticular canaliculi contain protoplasmic processes derived from the hypodermis, as Nordenskiöld found to be the case in *I. ricinus*. In preparations stained by the ordinary methods this structure of the cuticle is very difficult to see, on account of the extreme transparency of the parts. Nordenskiöld[4] recommends Golgi's Silver-Chromate impregnation method

[1] Nordenskiöld, E. (1908), pp. 657–658.

[2] *Haemaphysalis punctata*, *Rhipicephalus appendiculatus* and *Amblyomma hebraeum*.

[3] Nordenskiöld, E. (1908), pp. 659–664; Pl. 26, fig. 8, and Pl. 28, figs. 16–18.

[4] Nordenskiöld, E. (1908), p. 660.

as eminently suitable for their demonstration, but a fortunate accident rendered this troublesome process unnecessary in our case. For some unexplained reason, some sections which had been stained in an alcoholic solution of Thionin showed a very finely granular reddish-brown precipitate deposited throughout the tissues, and the cuticular canaliculi were most perfectly defined.

### The alimentary canal.

For purposes of anatomical description, the alimentary canal may be conveniently divided into the following successive portions:

| *Fore-gut.* | *Mid-gut.* | *Hind-gut.* |
|---|---|---|
| 1. The Buccal Cavity, with the Salivary Glands and Ducts. | 4. The Stomach, with its coecal appendages. | 7. The Anal Canal and Anus. |
| 2. The Pharynx. | 5. The Rectum (*tube communicatif* of Blanc). | |
| 3. The Oesophagus. | 6. The Rectal Sac, with its appended Malpighian Tubules. | |

### The Buccal Cavity.

Part I, Plate V, figs. 16–18; Plate VI, fig. 21.

The chitinous structures of the capitulum which enter into the formation of the buccal cavity have already received attention in the first portion of this paper (Part I, pp. 42–43). As there shown, the chelicerae and hypostome, by their apposition, form a long narrow tubular channel, the *buccal canal* (*buc. can.*), which terminates posteriorly behind the base of the hypostome in a cavity which is much flattened in the horizontal plane, but of considerable width from side to side. This space is the true *buccal cavity* (*buc. cav.*). Its roof is formed by the backward continuation of the cheliceral sheaths (*s. ch.*), while its floor is a direct continuation of that portion of the hypostome which forms the floor of the buccal canal and the hypostomal gutter (*h. g.*). The roof and floor meet and fuse together at the posterior limit of the buccal cavity and along its lateral margins, leaving the anterior portion open to the buccal canal. In the floor of the cul-de-sac thus formed, the pharyngeal orifice (*o. ph.*) opens, and the only other openings into the buccal cavity are situated at its postero-lateral angles, where the ducts of the salivary glands (*d. sal.*) debouch. In transverse sections through

the anterior portion of the basis capituli, the buccal cavity is seen to occupy a central position (see Pt. I, Pl. V, fig. 17). It is supported in front by its continuity with the buccal appendages, and laterally by the stout chitinous struts which are formed by the internal basal portions of the hypostome and palps. The roof of the buccal cavity is continued backwards within the cavity of the basis capituli in the form of a broad chitinous plate—the *subcheliceral plate* (*sub ch. p.*)—the dorsal surface of which is applied throughout its extent to the ventral surfaces of the dilated basal portions of the cheliceral shafts. The subcheliceral plate is very thin in its median portion, but acquires rigidity from its stoutly thickened lateral margins, which thickening continues directly into the chitinous struts alluded to above. The subcheliceral plate serves a dual purpose. It forms a smooth surface on which the bases of the chelicerae slide in the movements of protrusion and retraction, the raised thickened margins helping to maintain the chelicerae in position. Its ventral surface affords a means of attachment for the *dorsal dilator muscles* of the pharynx. The posterior margin of the subcheliceral plate like the lateral margins is thickened, and is also indented by a broad shallow notch.

The pharyngeal orifice is situated in the middle of the floor of the buccal cavity at the posterior extremity of the hypostomal gutter (*h. g.*) (see Part I, Pl. VI, fig. 21, *o. ph.*). The opening is guarded above by a tongue-like cuticular flap (*tg.*) formed from a forward continuation of the floor of the buccal cavity, an arrangement which appears to prevent the direct access of the saliva to the pharynx during the operation of sucking. Samson[1], in her description of the buccal cavity of *I. ricinus*, refers to the portion of the cavity lying above the tongue-like process—the cul-de-sac into the posterior angles of which the salivary ducts open—as the "salivary cavity" (Speichelhöhle), and includes, as part of the buccal cavity, the space below the tongue-like process, which is actually the posterior end of the hypostomal gutter, where it forms the upper part of the pharyngeal orifice. In Fig. 16 (Part I, Pl. V), the space which appears between the buccal cavity and the roof of the pharynx, is the continuation of the body cavity into the base of the tongue-like process, and has no communication with the alimentary canal.

[1] Samson, K. (1909 *a*), p. 192.

### The Salivary Glands.

Plates XIV, XV and XVI, *gl. sal.*; Plate XVII, figs. 5 and 6, Text-fig. 1.

These organs consist of a single pair of large, elongate, cream-coloured, glandular masses which occupy a ventro-lateral position in the anterior half of the body-cavity. They are situated immediately above the coxae of the first three pairs of legs and are almost entirely covered above by the anterior coecal lobes of the stomach, with which they are in contact. In the active state, they may extend, backwards and outwards, so as to reach almost to the spiracles, but it must be noted that their size depends to a great extent upon the condition of the tick. In the resting phase after engorgement, they may assume quite insignificant proportions, though they rarely show the extreme degree of degeneration which is exhibited by the *Ixodidae*.

Each gland consists of grape-like clusters of alveoli, loosely aggregated into irregular lobules, which surround the main salivary duct (*d. sal.*). This main duct, which commences near the posterior limit of the gland, receives numerous secondary ducts which enter it in a very oblique manner, one from each of the lobular masses. The alveoli are closely clustered on these secondary lobular ducts, each opening by a separate short efferent duct which usually enters the lobular duct directly, but occasionally may unite with the efferent ducts from one or two other alveoli, to enter the lobular duct collectively.

Between the alveoli a sparse connective tissue frame-work is developed, which is not sufficient, however, to give more than a small degree of coherence to the gland.

A careful examination of the gland leads to the recognition of two types of alveoli. These may be distinguished in the fresh gland, while still *in situ*, under the dissecting microscope, but after treatment with a fixing reagent such as dilute acetic acid, the two types become strikingly apparent. The first type forms the greater mass of the gland; the second type is limited to a mass of alveoli which are situated in the anterior half of the gland on the mesial side of the main salivary duct, a little towards the dorsal surface (see Text-fig. 1 B). In longitudinal sections of the entire gland, both types of alveoli are readily defined, but in transverse sections passing through the posterior half of the gland only, alveoli of the first type appear alone.

Apart from differences in size and the fact that the main ducts are generally somewhat flattened in section, the salivary ducts show a complete uniformity, in the principle of their structure, throughout the

gland. The outer wall of the duct consists of an epithelial layer, the individual cells of which are not defined, but the cellular nature of which is evident from the presence of numerous small ovoid nuclei which are embedded in the pale, very finely granular protoplasm. The most remarkable feature of the salivary ducts is the spiral thickening of the inner portion of the duct wall. The entire lumen of all the ducts possesses a chitinous lining continuous with the cuticle of the buccal cavity, and which extends to the very mouths of the gland alveoli. This cuticular lining shows a spiral thread-like thickening, an appearance

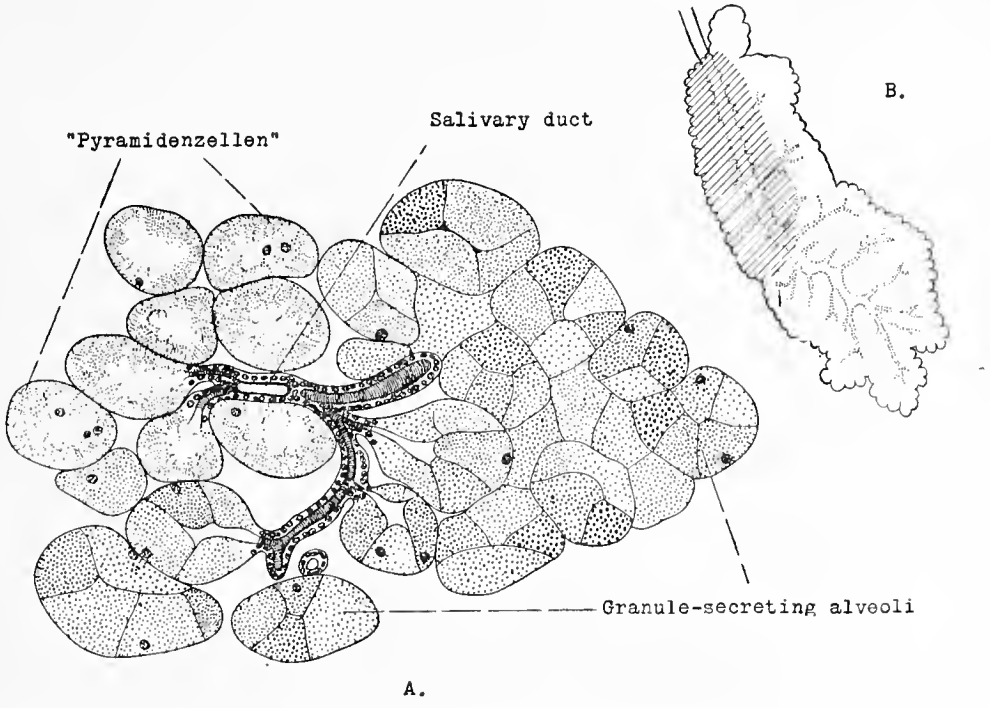


Fig. 1. *Argas persicus* ♀. A. Transverse section of the anterior half of the right salivary gland, showing the relative positions of the two types of alveoli. ×320 diam. B. The entire gland from the dorsal aspect. The shaded area indicates the situation of the alveoli of the *second type*—the "Pyramidenzellen" of Samson.

which has led the majority of observers who have described the structure, to compare it with that of the typically spirally-thickened air-tubes of tracheate Arthropods. Nordenskiöld[1], however, has objected to such a comparison, on the ground that the spiral thickening of the salivary ducts is not a part of the cuticular lining, but is actually embedded in the protoplasmic epithelium and, therefore, lies entirely without the

[1] Nordenskiöld, E. (1908), pp. 648–649.

chitinous layer. In longitudinal sections of the salivary duct, the spiral thickening certainly is seen to lie on the epithelial surface of the chitinous lining, but it is extremely difficult to decide that it is a separate structure. Nordenskiöld[1] has observed that in torn sections, where the salivary duct is ruptured, the spiral thread comes away with the cell-protoplasm and not with the chitinous cuticle, as is the case with a trachea. On the other hand, we have noticed that specimens which have been cleared in potash, and in which, therefore, all but the chitinous structures are dissolved, the spiral thickening of the salivary duct is still clearly visible on the outer surface of the chitinous lining. Nordenskiöld suggests that the spiral thickening is a contractile thread which functions as a regulator of the salivary duct, but, in the absence of further evidence, we are inclined to consider the structure as a spiral thickening of the chitinous cuticle which projects on the epithelial surface and which serves to maintain the patency of the ducts.

As already mentioned, the cuticular lining of the salivary ducts extends quite to the mouths of the alveoli, and, in the case of the alveoli of the first type, just at its termination within the mouth of each alveolus, the lining is slightly thickened and frequently shows a few longitudinal folds. This is, without doubt, the appearance which Nordenskiöld interpreted as a valvular structure[1].

The main salivary ducts become free of the glands just before they pass through the capitular foramen (see Pl. XIV, *f. c.*). Within the basis capituli they converge slightly as they run forwards beneath the lateral margins of the subcheliceral plate towards the buccal cavity, into the postero-lateral angles of which they open. (Part I, Pl. V, figs. 18–20, and Pl. VI, fig. 21, *d. sal.*)

Reference must now be made to the histological structure of the two types of alveoli which have been previously alluded to. Bonnet[2] was apparently the first to recognise the two forms as they occur in *I. ricinus*, and Samson[3] subsequently confirmed his observation; Nordenskiöld, however, makes no mention of any distinction although one of his figures[4] gives convincing evidence of their existence.

The greater mass of the gland is formed of alveoli of what, up to the present, have been referred to as the *first type*, and it can only be to this type that Nordenskiöld's very detailed description applies.

Each alveolus of this first type (Pl. XVII, fig. 5) is roughly pear-shaped, the smaller end being the place of attachment to the efferent

[1] Nordenskiöld, E. (1908), p. 649.
[2] Bonnet, A. (1907), pp. 51–58.
[3] Samson, K. (1909 *a*), pp. 202–205.
[4] Nordenskiöld, E. (1908), Pl. 27, fig. 12.

duct. In sections of the entire tick, the individual alveoli usually present a more or less polygonal outline, due to mutual pressure; in dissections, however, they generally appear rounded and quite loosely aggregated. Sections of the gland show each alveolus to be enclosed by a delicate basement membrane upon which the bases of the secretory cells rest. The latter are large cells, more or less pyramidal in form, whose apices are directed towards the centre of the alveolus where they surround a small lumen which communicates directly with the efferent duct. At the time of maximum activity, the secretory cells are so distended with secretion that they encroach upon and almost obliterate the lumen. The cytoplasm of these secretory cells is coarsely reticulate, and its appearance depends upon the stage of activity of the cell. The nuclei are rounded, of moderate size, and are usually situated near the base of the cell. In mature cells, they are generally invisible on account of the dense mass of deeply-staining ferment granules with which they are surrounded. In the same alveolus, secreting cells may be found in all stages, from the cell in which the secretory product is just commencing to accumulate, to the cell which is literally bursting with its secretion, and finally, cells appear, here and there, whose limiting membrane has ruptured and the contents of which have poured out into the lumen of the alveolus, leaving little else behind, other than the coarse protoplasmic reticulum, which stands out, clearly defined, in the now pale-coloured exhausted cell. When secretion recommences, such a cell becomes progressively darker and more opaque, and spherical droplets or granules of the secretion appear, in due course, in the meshes of the reticulum. As the process continues, the granules increase in size, and become more and more susceptible to the staining action of the basic dyes. By the time the final stage is reached, the cell may be so crowded with these deeply-staining granules, that all detail of structure is completely obscured, the cell appearing in sections as a densely stained wedge-shaped sector of the alveolus. From their structure, these secretory cells are undoubtedly homologous with the "Mündungszellen," as Nordenskiöld termed them[1], of the salivary gland of *I. ricinus*. The "Funduszellen" of this author are apparently absent in *A. persicus*, as von Künssberg[2] has found to be the case in *Ornithodorus moubata*. Samson, however, has recognised in the coxal glands of *O. moubata*, structures which she considers to be the homologues of the "Funduszellen" of the salivary glands of *I. ricinus*, but, as will be shown subsequently, in

[1] Nordenskiöld, E. (1908), p. 647.
[2] Künssberg, K. von (1911), p. 265.

dealing with the anatomy of the coxal glands in *A. persicus*, our observations do not agree with her discoveries in this direction.

Heller[1] observed two types of alveoli in the salivary gland of *A. persicus* and there is no doubt that he alludes to, and also figures, both forms. The alveoli of the second type were first described in detail by Bonnet[2], who, on account of their resemblance in structure to the venom-secreting cells in the poison-glands of certain snakes, concluded that they fulfilled a corresponding function, and therefore, termed them *glandes à venin*. Samson has recognised these alveoli, which she terms "Pyramidenzellen," in *I. ricinus*[3] and also in *O. moubata*[4]. In *I. ricinus*, they are found distributed on the branches of the salivary ducts throughout the gland, while in *O. moubata*, as we have also found to be the case in *A. persicus*, these alveoli—the "Pyramidenzellen" of Samson—are collected together in a single mass on the mesial side of the main salivary duct at the anterior end of the salivary gland.

The alveoli of the second type differ from those of the first type—the granule-secreting alveoli described above—in the fact that they are appreciably smaller in size and instead of being borne on more or less long and branching efferent ducts, they are closely applied to the main salivary duct, with which they communicate by a short efferent duct which, however, shows no difference in structure from the efferent ducts of the granule-secreting alveoli. In sections, like the granule-secreting alveoli, each appears enclosed by a delicate basement membrane, but the secretory elements are markedly different. Both Bonnet and Samson described these alveoli as being unicellular. It is true that the individual cells are not defined by a cell membrane, but the number and arrangement of their nuclei surely indicate their existence. In place of the coarse reticulation and granulation of the cytoplasm of the granule-secreting cells, these cells show a delicate fibrillar structure, the threads of which run from the basement membrane towards the lumen of the alveolus. The free margin of the cells is quite indefinite and at this point the fibrillar structure appears to merge into a very coarse reticulum, the meshes of which communicate, without the interposition of a limiting membrane, with the lumen of the alveolus. The nuclei of these alveoli are smaller and fewer in number than in the granule-secreting alveoli, and are frequently situated so far from the bases of the cells as to appear to be almost free in the lumen. The secretion is apparently a clear homogeneous liquid; in any case, large

[1] Heller, C. (1858), p. 308.
[2] Bonnet, A. (1907), pp. 56–58.
[3] Samson, K. (1909 *a*), p. 203.
[4] Künssberg, K. von (1911), p. 264.

ferment-granules as seen in the alveoli of the first type, are never visible in the "Pyramidenzellen."

Regarding the functions of the two types of alveoli which constitute the salivary gland of *A. persicus*, we, never having experimented with the salivary secretion, are unable to make any definite statement. The reader must be referred to the special work on this subject embodied in papers by Sabbatani[1], von Künssberg[2], and Nuttall and Strickland[3]. Nordenskiöld discusses the probable functions of the secretions of the "Funduszellen" and the "Mündungszellen" respectively[4], but as his remarks are based on comparisons of the structure of these elements with similar structures in other animals, the function of which is definitely known, they are merely conjectural. There is, however, no doubt that one of the products of secretion of the salivary glands is an anticoagulin. Whether a digestive ferment is also secreted, remains unproven, and the existence of a venomous principle in the saliva, the presence of which has been surmised by many writers on the subject of tick biology, is still a matter of doubt.

## The Pharynx.

Part I, Text-fig. 2; Plate V, figs. 16–20; Plate VI, fig. 21;
Part II, Plates XV and XVI, fig. 1; Text-figs. 2 and 3.

The pharynx is that portion of the alimentary canal which connects the buccal cavity with the oesophagus, and, in common with the majority of the *Arachnida*, it constitutes a powerful pumping organ which sucks in the fluid nutriment—blood in the case of ticks—and forces it into the stomach.

The greater part of the organ is contained within the basis capituli but its posterior end protrudes for a short distance through the capitular foramen into the body cavity. Its anterior end is continuous with the buccal cavity, in the floor of which, as has been already seen, the pharyngeal orifice opens. The pharynx of *Argas persicus* differs from that of the Ixodid ticks in that the diameter of its middle portion greatly exceeds that at the extremities, thus giving it a short "torpedo" form. From the middle of its length the organ tapers in a curving manner towards either extremity, becoming much constricted at the

[1] Sabbatani, L. (1898). [2] Künssberg, K. von (1911).
[3] Nuttall, G. H. F., and Strickland, C. (1908).
[4] Nordenskiöld, E. (1908), p. 653.

anterior end, where the direction of its lumen turns upwards and forwards to enter the buccal cavity. The walls of the pharynx are composed of a hypodermal layer and a stout chitinous lining, the latter representing the cuticle, here specially developed, which is common to the whole course of the *fore-gut*.

The real nature of the organ becomes apparent from the examination of its transverse section (see Text-figs. 2 and 3). It is then seen that the walls are thrown into a number of sharp regularly-disposed longitudinal folds, arranged in such a manner as to give it a tri-radiate cross-section. Two of the radii form the dorsal part, and a single median ventral radius completes the figure. The extremity of each radius is divided by a deep infolding of the pharyngeal wall, thus

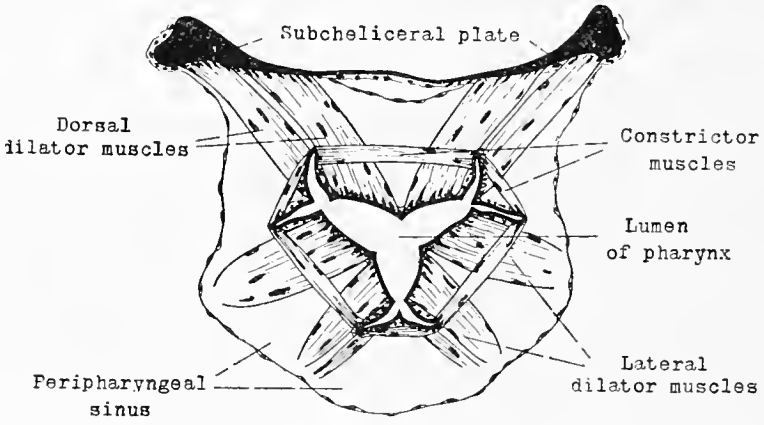


Fig. 2. *Argas persicus* ♂. Transverse section through middle portion of pharynx. As the lateral dilator muscles pass obliquely backwards, their attachments to the wall of the basis capituli do not appear in the section. × 130 diam.

giving the section of the pharynx a form which may be compared to a Maltese cross with three arms.

From the walls of the pharynx, regular series of muscle-bands radiate on all three sides of the organ, the muscles of the dorsal surfaces being attached at their distal extremities to the under surface of the subcheliceral plate, and the lateral muscles to the wall of the basis capituli. In addition to these, a second series of smaller muscle bands are seen to connect the extremities of each of the radii with one another, and also the bifurcated extremities of each radius. These muscle bands, which thus form a complete ring surrounding the entire circumference of the pharynx, alternate in a perfectly regular manner with the above-mentioned radiating bands.

If, now, the form of the pharynx is considered, it is seen that it is such as to allow a great range of variation in the capacity of the organ, accordingly as its walls are in contact or separated. Separation of the pharyngeal walls, and consequently dilation of the pharyngeal cavity, is brought about by the larger radially-disposed muscle bands—the *dilator muscles* of the pharynx—and by the contraction of the smaller circularly-disposed muscles—the *constrictor muscles* of the pharynx—the pharyngeal walls are brought into apposition and the internal cavity is practically obliterated (see Text-fig. 3).

The function of the pharynx being obvious, it now becomes necessary to see what provision is made to ensure that the ingested blood is forced in one direction, viz. towards the stomach. We have made repeated

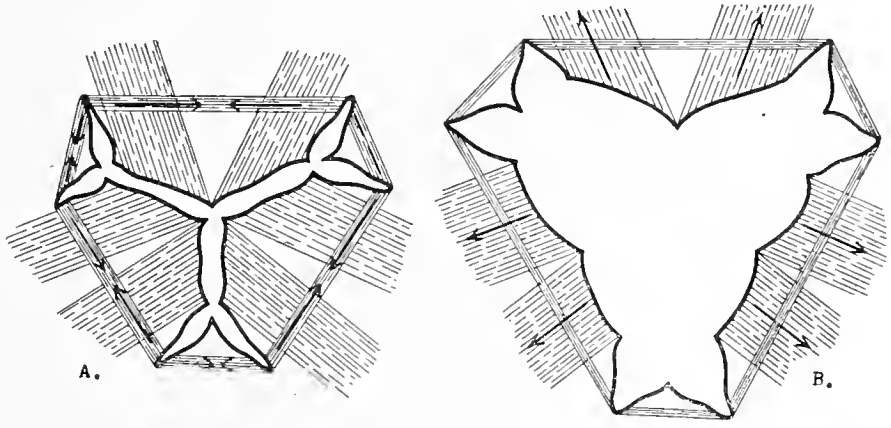


Fig. 3. Diagrammatic representation of the cross section of the pharynx :—A, in a state of contraction; B, in a state of dilation. The action of the muscles is indicated by the arrows.

efforts to discover a valvular structure at the anterior pharyngeal orifice, but without success. The lumen of the organ, as already stated, is constricted at this point, but nothing of the nature of a valve can be recognised. It would, therefore, appear that the *vis-a-tergo* of the blood in the buccal canal and buccal cavity is greater than that of the contents of the oesophagus and stomach, so that when the pharynx contracts, its contents follow the direction of least resistance, *i.e.* through the oesophageal orifice. Having reached the stomach, regurgitation of the ingested blood is prevented by the peculiar structure of the proximal end of the oesophagus. As will be shown later, the oesophagus is telescoped for a short distance into the cavity of the stomach, forming

what Christophers[1] has termed the *proventricular fold*, and this structure evidently fulfils a similar function to the corresponding structure which is found in many suctorial *Hexapoda*.

### The Oesophagus.

Part I, Text-fig. 2; Part II, Plates XIV, XV and XVI, *oes.*

The oesophagus is a soft-walled tube of small calibre, which connects the pharynx with the stomach. Its course is not quite direct, for, immediately at its commencement at the posterior end of the pharynx, it bends abruptly downwards and slightly forwards; then almost immediately it again turns backwards, forming a short **S**-shaped bend before continuing its course through the brain, entering this organ near the anterior end of its ventral surface, and emerging on the middle of the dorsal surface to enter the stomach almost directly. At its proximal extremity, the oesophagus protrudes for a short distance into the lumen of the stomach, where it forms the previously mentioned *proventricular fold.*

In cross-section, the oesophagus is circular. Its walls consist of an epithelial layer of irregularly columnar cells with small nuclei, surrounded by a thin muscular layer composed for the most part of circularly arranged fibres, a few longitudinal strands of muscle fibre appearing in the peripheral portions. The lumen of the oesophagus is lined with a delicate chitinous intima, which, together with the underlying epithelium is thrown into shallow longitudinal folds. The proventricular fold is composed of the same elements as the rest of the oesophagus, but the epithelial layer, which becomes enormously thickened, is here composed of elongate columnar cells, which merge abruptly into the epithelium of the stomach wall. The chitinous lining of the oesophagus is continued over the internal surface of the proventricular fold but terminates at the point where its epithelium joins that of the stomach.

### The Stomach.

Plates XIV and XV; Plate XVI, figs. 1–6: *st., cc. al.*

The stomach with its coecal appendages forms the greater part of the alimentary canal and is, indeed, the bulkiest organ in the body of the tick. It consists of a median portion—the stomach proper (see

[1] Christophers, S. R. (1906), p. 31.

Pls. XIV and XV and Pl. XVI, fig. 5, *st.*)—which occupies the central part of the body cavity, extending from the dorsal surface of the brain in front, to a point a short distance anterior to the level of the anus. In the adult stage, the stomach overlies and, together with its coecal appendages, almost entirely conceals the genital organs from the dorsal aspect. At its anterior and posterior extremities, the stomach is bifurcated, giving rise to four great lobes which occupy respectively the four quarters of the body. For purposes of distinction, these coecal lobes may be termed *antero-lateral* (*right* and *left*), and *postero-lateral* (*right* and *left*). The deep notch which separates the antero-lateral and postero-lateral coecal lobes of each side runs obliquely forwards from the margin of the body to the anterior end of the stomach (see Pl. XIV), and through this notch certain coxal and other muscles pass upwards to their places of insertion into the dorsal cuticle. Through this great notch also pass the great postero-dorsal tracheal trunk and the Malpighian tubules after their dorsal course over the postero-lateral coecal lobe. Two other deep notches are worthy of mention. The first of these is that which divides the postero-lateral lobe into two nearly equal portions. This notch contains the postero-accessory dorso-ventral muscles, and through it the Malpighian tubules reach the dorsal surface of the alimentary canal. The second notch is the median unpaired division between the right and left postero-lateral coeca, through which pass the postero-median dorso-ventral muscle columns. The antero-lateral coecal lobe is generally divided by deep marginal notches into two or more parts, but these notches are not so constant in their arrangement as those described above. They afford a means of passage for the first three pairs of abductor muscles of the coxae, the fourth pair passing with the adductor muscles of the coxae through the notch between the antero-lateral and postero-lateral coecal lobes. Further reference to these muscles will be made in a subsequent part of this paper dealing with the musculature. The ultimate terminations of the alimentary coeca form a series of sacculated pouches, which reach almost to the periphery of the body, the shallower notches which divide these marginal pouches accommodating the marginal dorso-ventral muscles.

The general appearance of the stomach depends upon the state of engorgement of the tick. The empty stomach, with its appendages, is pale in colour, but assumes a deep purple-red hue after engorgement, which, as digestion proceeds, changes to chocolate and then to dark brown. In old specimens in which digestion is complete, the entire organ becomes collapsed and flattened and presents a dirty greyish-black

colour, derived from the undigested detritus which remains within its lumen. After engorgement, the coecal lobes become so distended and crowded together, that it is a difficult matter to recognise the different parts.

The oesophageal opening of the stomach is situated at the anterior end of its ventral portion, immediately behind the point where the latter bifurcates to form the antero-lateral lobes. The rectum originates in a corresponding position at the posterior end of the stomach, where it commences as a funnel-shaped depression of the ventral wall (see Pls. XIV and XV, *t. c.*).

The stomach wall consists of two layers—an inner epithelial layer and a very thin outer muscular layer—the latter appearing to be more or less embedded in the delicate homogeneous *membrana propria* upon which the bases of the epithelial cells rest. The epithelial structure varies very considerably in the different phases through which it passes, prior to and after engorgement. In unfed individuals the epithelial cells are cylindrical, and though they may vary somewhat in size, they show a complete uniformity in structure throughout the entire viscus. After engorgement, the epithelium becomes thicker, and here and there individual cells become elongated so as to protrude into the lumen of the organ beyond the general surface of the stomach wall (see Pl. XVII, fig. 2). These pseudopod-like protrusions of the epithelium assume a more or less spherical form, but always remain attached to the basement membrane by their now pedunculate bases. The nuclei of these epithelial cells, which, in unfed individuals, are situated near the basement membrane, move towards the free margins of the cells and take up their position in the protruded spherical extremities. The cell contents also undergo considerable changes. Before engorgement, the cytoplasm is finely granular and, as mentioned above, the nuclei are situated near the bases of the cells. After engorgement, and while active digestion is in progress, the proximal portions of the cells develop a very striking reticulate structure, and the distal portion of each cell, more particularly those which have elongated to form the pseudopodial protrusions, become densely packed with large spherical granules which are deeply stained by the acid dyes. Whether these spherical granules represent the secretory product of the epithelial cells or not, is doubtful, for after digestion has proceeded for a time, similar granules appear within the body cavity, suspended in the blood-serum, and are even found ingested by the amoeboid blood corpuscles. This seems to indicate that the acidophile granules are the actual nutrient products

elaborated from the contents of the stomach, and passed on in this form to the coelomic spaces surrounding the gut, to be carried to the different parts of the body by the blood stream. In *Ixodes ricinus*, Nordenskiöld[1] has actually observed that the epithelial cells of the stomach and its coecal appendages put forth pseudopodial protrusions from their basal surfaces, which ultimately rupture and discharge their elaborated nutrient products into the coelom. We have never seen this phenomenon in *Argas persicus*, but that it does occur is probable, from the fact already mentioned, that during the digestive process large numbers of spherical granules, which appear to be identical with those contained within the epithelial cells, are found in the coelomic spaces.

The foregoing description of the gastric epithelium applies to both the stomach and its coecal appendages; the two parts do not show any marked difference in structure, except that in the central stomach the epithelium does not show such great activity during the period of digestion as does that of the coecal appendages.

An interesting point in connection with the digestive process in *A. persicus*, is that the large crystals, which appear in the ingested blood mass in Ixodid ticks, have never been observed.

After digestion is completed, the gastric epithelium undergoes a process of degeneration; the cells become packed with dirty brownish-black granules of excretory matter, and the details of their original structure is lost. The lumen of the mid-gut still retains a mass of similar granules embedded in a homogeneous matrix of a paler dirty-brown colour. Whether the walls of the alimentary coeca and stomach undergo a complete histolysis and regeneration between each moult, in *A. persicus*, we cannot at present state.

The various phases through which the tick passes in the course of its life, makes the study of histological detail no easy matter. As Nordenskiöld[2] observes, the transformations are very difficult to follow in a sequent manner, and in order to investigate the subject properly, it would be necessary to carry out a lengthy and troublesome research with this special object in view, using material raised under such conditions that its history was definitely known.

The muscular layer of the stomach wall does not appear to be so highly developed in *A. persicus* as that observed by Nordenskiöld[3] in *I. ricinus*. The muscle bands form an irregular interlacing network which extends over the entire surface of the organ, but is not differentiated

[1] Nordenskiöld, E. (1908), p. 645. [2] Nordenskiöld, E. (1908), p. 643.
[3] Nordenskiöld, E. (1908), pp. 671–673.

into longitudinal and transverse bands. Many of the fibres anastomose, and in those cases where the ends are free, the termination of the fibre is split up, fan-wise, into a number of fibrils which lose themselves in the basement membrane supporting the epithelial layer. The muscle bands, and particularly the terminal fibrils, show a regular transverse beading, the only evidence of their transversely striated nature, but this feature is not always visible, its successful demonstration being largely dependent upon the method of fixation and staining used. Fig. 3 (Pl. XVII) was drawn from a portion of the wall of one of the alimentary coeca removed in the fresh state, opened out and spread on a glass slip, fixed with Carnoy's Fluid and stained with Ehrlich's so-called "tri-acid" mixture (Orange G., Säurefuchsin and Methyl Green). The nuclei which appear in the preparation are those of the epithelial cells which still remained attached to the basement membrane, the majority having been intentionally removed by gently dabbing the epithelial surface with a fine soft brush prior to staining.

In the living tick, peristaltic contractions of the alimentary coeca may be observed through the translucent integument, the movements being particularly active immediately after engorgement.

### The Rectum.

Plates XIV and XV, *t. c.*

We have applied the term "*rectum*" to the narrow, comparatively short tube which establishes communication between the stomach and the *rectal sac,* but, as both the rectum and rectal sac of the *Ixodoidea* are derived from the *mesenteron,* the terminology is not strictly correct. In the literature on the subject, this portion of the alimentary canal of the tick has received many names. Heller[1] called it the *intestine* (*Darm*): Nordenskiöld[2] refers to it as the *pylorus*: Bonnet[3] and others deny its existence altogether, the former describing the stomach with its appendages as *un organe aveugle sans communication avec le "rectum" ou vésicule excrétice.* Blanc[4], in a paper dealing solely with this subject, terms it the *tube communicatif* and applies the term *rectum* to the *rectal sac.* For fuller information the reader is referred to his paper, which contains a very complete account of the comparative anatomy of this portion of the alimentary canal in the *Ixodoidea.*

[1] Heller, C. (1858), p. 307.
[2] Nordenskiöld, E. (1908), p. 646.
[3] Bonnet, A. (1907), pp. 61–66, 83.
[4] Blanc, G. (1910).

The rectum commences as a funnel-shaped depression of the posterior portion of the ventral wall of the stomach, immediately anterior to the bifurcation of the latter organ from which the postero-lateral coeca arise. It then continues, downwards and backwards, as a narrow cylindrical tube to the anterior portion of the under surface of the rectal sac, into which it opens at this point. In sections, the walls of the funnel-shaped upper extremity of the rectum are seen to be identical in structure with that of the stomach, but from this point downwards, the cells lose their individuality, the rectal wall here consisting of a finely granular homogeneous protoplasm in which is embedded a layer of crowded nuclei. The lumen of the tube, especially in its lower portion, is generally completely obliterated, and it does not appear to become patent until the final stages of the digestive process is reached. Not until the gastric epithelium has degenerated, do excretory products from the stomach appear in the rectal sac, and as the rectum is the only means of communication between the two organs, the excretory granules derived from the alimentary coeca and stomach, which do ultimately appear, mingled with the secretion of the Malpighian tubules in the rectal sac, must have traversed the rectum.

### The Rectal sac.

Plates XIV, XV and XVI, fig. 6; Plate XVII, fig. 4, *s. r.*

The rectal sac is a large vesicle, with delicate transparent walls, into which the rectum and the Malpighian tubules open, and from which a short canal communicates with the anus. It is usually rendered very conspicuous by reason of its opaque white contents. The body of the sac lies below the ovary in the female and the testis in the male; its lower surface is in contact with the ventral body wall. Just behind the anus, on either side of the series of postero-median dorso-ventral muscles, the rectal sac is continued backwards for a variable distance as a pair of large diverticula, to which its bilobed appearance is due. The walls of the sac are excessively thin and unless the greatest care is taken, they are generally ruptured in making dissections of the entire animal, the opaque white granular contents—the secretion of the Malpighian tubules—being thereby scattered in the body cavity. Its structure most closely resembles that of the Malpighian tubules, though the epithelial lining is considerably reduced in thickness and has apparently no secretory function. The cytoplasm of the cells is so sparse that the nuclei actually exceed in their diameter the thickness of the

wall of the sac and bulge out into the lumen. As mentioned in the previous section, the excretory products derived from the alimentary canal do not enter the rectal sac until the final stages of digestion are reached and in consequence the contents, as usually seen, consist entirely of the secretion of the Malpighian tubules.

### The Malpighian tubules.

Plates XIV, XV and XVI, *t. mpg.*; Plate XVII, fig. 7.

These structures, two in number, enter the rectal sac on its ventral side, close to the median line, on either side of the rectal aperture (see Pl. XV, *o. t. mpg.*).

In appearance, the tubules are opaque creamy-white, the colour being due to the contents, as was seen to be the case with the rectal sac. They are generally slightly flattened in cross-section, unless distended with secretion, and their diameter varies somewhat in different parts, the average diameter being about 0·1 mm.

The Malpighian tubules follow a somewhat complex course through the body of the tick and extend almost to the anterior end of the body. From their point of origin at the rectal sac, the two tubules run for a short distance backwards, each following the lateral contour of the rectal sac. Before reaching the posterior margin of the latter, however, they suddenly turn forwards and upwards, passing through the deep notch between the two subdivisions of the postero-lateral lobes of the stomach, and emerge on the dorsal surface of the gut. After crossing the neck of the postero-lateral lobe, the tubules return to the ventral part of the body cavity once more, passing downwards through the notch which separates the antero-lateral and postero-lateral lobes of the stomach. From this point they continue their forward course for a short distance alongside the genital canals in the adult, and then turn outwards beneath the salivary glands, along the lateral margins of which they run towards the anterior end of the body, where they terminate blindly at the level of the basis capituli, the blind extremities being situated, as a rule, in or near the camerostomal folds.

The Malpighian tubules are occasionally coiled and reflexed on themselves, but not to nearly the same extent as those of the Ixodid ticks, in which, though the general arrangement is similar to that described above, the whole system is complicated by the numerous secondary convolutions which they exhibit. On account of the non-development of these secondary convolutions, the Malpighian tubules of

*Argas persicus* are relatively shorter than those of the *Ixodidae*, a fact which is doubtless explained by the different habits of feeding.

In their histological structure, excepting one or two details, the Malpighian tubules closely conform to Nordenskiöld's[1] description of those of *Ixodes ricinus*. Each tubule is formed entirely of large secretory cells, the broad flattened bases of which rest on a delicate basement membrane. The free surfaces of the secretory cells are generally rounded and bulge out, more or less, into the lumen of the tubule, the latter thus appearing, in transverse sections of the tubule, to send narrow diverticula between adjacent cells. The cytoplasm of the secretory cells is finely granular, but shows a very definite reticulum upon which innumerable secretion-granules are scattered. The nuclei, of moderately large size, are usually ovoid in shape and are situated near the bases of the cells. The chromatin is distributed in sharply defined angular masses, and each nucleus contains one or more nucleoli. Dissolution of the nuclear membrane may occasionally be observed, in which case the nucleoli and small rounded masses of chromatin are found lying free in the cytoplasm.

The free margins of the secretory cells generally show a narrow zone of homogeneous cytoplasm from which the reticulate structure is absent, but which sometimes exhibits a faint striation, the parallel striae running in a direction at right angles to the free surface of the cell (the *Stäbchensaum* of Nordenskiöld[2]).

The secretion of the Malpighian tubules consists of a clear colourless fluid in which are suspended the singular, highly-refractive concretions which are so familiar to all who have examined microscopical preparations of ticks. These concretions are found scattered at intervals along the whole course of the tubules, but in the rectal sac they appear in enormous numbers, and ultimately the sac is absolutely packed with them. They are never actually found within the secretory cells, and evidently crystallise out from the fluid secretion which the cells pour out into the lumen of the tubule. The Malpighian concretions, which appear yellowish by transmitted light, each show a very definite concentric striation, and in many cases, fine radiating fissures extend from the dark point, which forms the centre of the concretion, towards the periphery. Double concretions, in which the laminar deposits appear to have taken place round two adjacent nuclei, are common.

As to the chemical nature of these concretions, it was supposed for

[1] Nordenskiöld, E. (1908), pp. 655–656.

[2] Nordenskiöld, E. (1908), p. 655, and Pl. 27, fig. 14.

a long time that they consisted of *uric acid* or *urates*. Pagenstecher[1] was the first to apply a chemical test with the object of establishing their constitution, and concluded that they were composed of *uric acid*. Kersey[2] is cited by Bonnet[3] as having identified their substance with *guanin*, a discovery which has since been confirmed by Bonnet.

Before leaving the subject of the Malpighian tubules, it should be observed that these structures in ticks are, in all probability, not homologous with those of the Malpighian tubules of the *Antennata*. In the latter class, the Malpighian tubules arise as invaginations of the wall of the *proctodoeum*, while in all Arachnida in which the subject has been investigated, they are derived from the *mesenteron*. The earliest reference to the subject, with which we are acquainted, is that of Loman[4] (cited by Warburton[5]), who showed that in spiders the Malpighian tubules open into the mid-gut and not into the rectum (hind-gut), and there is reason to believe that structures homologous with the Malpighian tubules of Insects are absent in the Arachnida, where their place is taken by the coxal glands, which are considered to be the true excretory organs.

Lankester[6], in discussing the same subject, cites Laurie[7], who found the Malpighian tubules of Scorpions are developed from the *mesenteron* and are, therefore, *hypoblastic* in origin, while in the Hexapoda, they are derived from the *proctodoeum* and are *epiblastic* in origin. On p. 205 of the same work, Lankester cites Korschelt and Heider[8], who point out that the hinder portion of the gut frequently acts in *Arthropoda* as an organ of nitrogenous excretion, in the absence of any special excretory tubules, and that the production of such coeca from its surface, in separate lines of descent, does not involve any elaborate or unlikely process of growth. Lankester sums up—"In other words, the Malpighian tubes of the terrestrial *Arachnida* are homoplastic with those of the *Hexapoda* and *Myriapoda*, and not homogenetic with them."

### The Anal Canal and Anus.

Pl. XV, *an.*; Pl. XVI, fig. 6, *an.*; Pl. XVII, fig. 4, *an. ap.*, *an. can.*

The anal canal is a very short wide laterally compressed tube which passes directly downwards from the ventral surface of the rectal sac to the anus, the upper opening of the tube being situated a short distance

[1] Pagenstecher, H. A. (1861), p. 34.
[2] Kersey (1872).
[3] Bonnet, A. (1907), p. 87.
[4] Loman, J. C. C. (1885–87).
[5] Warburton, C. (1909), p. 331.
[6] Lankester, E. R. (1904), pp. 204–205.
[7] Laurie, M. (1890).
[8] Korschelt, G. and Heider, K. (1892).

posterior to the rectal opening. It is formed of an epithelium of more or less columnar cells which are apparently similar in nature to the hypodermal cells. At the anal orifice this epithelium merges gradually into the general hypodermis of the ventral body-wall; at the upper extremity of the anal canal it extends for a short distance into the rectal sac. The anal canal is lined within by a very thin chitinous cuticle which is continuous with the general integumental chitin and extends as far as the opening into the rectal sac, where it thins out and disappears.

The structure of the anus has already received attention in Part I of this paper (p. 32), but one or two details of internal structure should be referred to.

The muscles of the anus consist of a single pair of obliquely inclined bands which extend from the upper part of the anal canal to the lateral margins of the anal valves. By their contraction, the lips of the anal aperture are everted, and the expulsion of the excreta is apparently brought about by the contraction of the dorso-ventral body muscles. The anal muscles, which Nordenskiöld[1] described in *Ixodes ricinus* as extending from the anal ring to the dorsal integument, are absent, and the large muscles which appear in his figure are evidently the foremost columns of the series of postero-median dorso-ventral body muscles. The true anal muscles are, however, represented in his figure, and appear to be identical, in their relations to the anal canal and the anal valves, with those of *Argas persicus*.

## The blood-vascular system.

Though more primitive than the circulatory system of some of the *Arachnida* (*Scorpionidae* and *Araneidae*), the blood-vascular system of *Argas persicus* does not appear to be quite so degenerate as the observations of some of the workers on the anatomy of the *Ixodoidea* would lead one to suppose. It is, however, generally known that in *Arthropoda* with a highly developed tracheal system, the peripheral portions of the blood-vascular system are correspondingly reduced, and in some cases even the heart may disappear; while in the case of Arachnida which show a concentration of the respiratory system, in the form of "book-gills," the heart and vascular arrangements are more complex and well-developed.

[1] Nordenskiöld, E. (1908), p. 647 and Pl. 27, fig. 13.

### The Heart.

Plates XIV, XV and XVI, *ht.*, Plate XVII, fig. 8.

The heart is a single-chambered vessel which is situated in the upper portion of the body-cavity immediately beneath the dorsal integument, a short distance posterior to the level of the spiracles. As seen from above, the organ appears as a small transparent flattened sac, resting on the dorsal surface of the stomach, and which in the living animal exhibits a rapid and regular pulsation. Its contour is more or less triangular, the angles being rounded off; and from the anterior angle—the apex of the triangle—arises a single large vessel, the *cephalic aorta* (*ao.*), which runs forwards on the surface of the stomach and disappears from view at the point where the latter organ divides to form the two antero-lateral lobes. The caudal artery which Nordenskiöld[1] identified in *I. ricinus* is not developed in *A. persicus*.

On account of the exceedingly delicate texture of its walls, and the fact that the organ is attached to the surrounding parts by strands of muscular and connective tissue, it is a matter of the greatest difficulty to remove the heart in its entirety; it is, therefore, most convenient to study its structure from serial sections of the entire animal. The walls of the heart (see Pl. XVII, fig. 8) are composed of a thin layer of homogeneous protoplasm, covered on both inner and outer surfaces by a delicate limiting membrane, within which the muscle fibres (*m. ht'.*) and numerous flattened nuclei are distributed. No cellular divisions can be recognised. In the ventral wall, situated a little towards the anterior end of the organ, two slit-like *ostia* (*os. ht.*) appear, one on either side of the median line; each ostium is guarded by a valvular flap (*v. os. ht.*) which is developed from the posterior margin of the opening and which protrudes into the lumen of the heart. The muscle fibres in the heart wall are transversely striated, and are, for the most part, disposed in a radial manner, extending from the central portion of both upper and lower walls towards the periphery of the organ. Other muscle fibres surround the margins of the ostia and extend to the valvular flaps which guard their orifices. In addition to the intrinsic muscles of the heart, other muscle strands (*m. ht.*) extend from the surface of the heart to the surrounding tissues. It would appear, from their arrangement, that the intrinsic muscles are the *contractor* muscles of the heart, and that the extrinsic muscles function as *dilators*.

[1] Nordenskiöld, E. (1909), p. 453.

### The Aorta and Peripheral Circulation.

The aortic wall shows a similar structure, on a reduced scale, to that of the heart; the muscle fibres, however, are much finer and more sparsely distributed, and the extrinsic fibres are absent altogether. As already mentioned, the aorta runs forwards along the dorsal surface of the stomach and turns downwards through the notch which divides

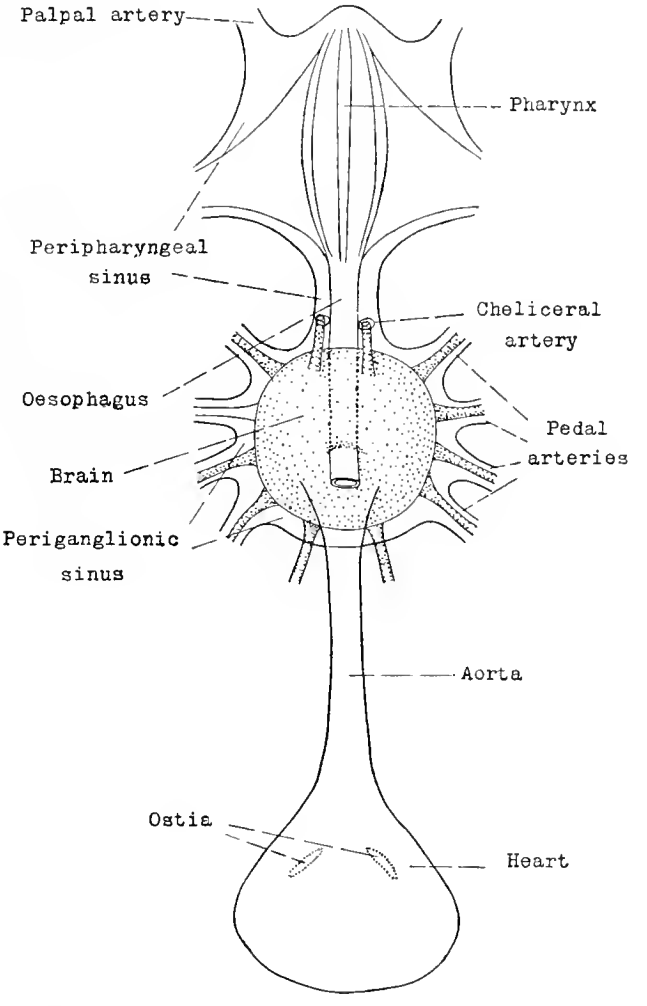


Fig. 4. *Argas persicus*. Diagram of the heart and arterial circulation of the adult. × 50 diam.

the antero-lateral lobes of the latter. On reaching the dorsal surface of the brain it opens into a large blood-sinus (see Pl. XV; and Pl. XVI, figs. 2 and 3, *ao., s. pg.*)—the *periganglionic sinus*—which appears to completely envelop the central nervous system. On either side of this

great sinus, coinciding in position with the origins of the pedal nerves, four arterial trunks open (see Text-fig. 4), and run outwards, one to each of the legs. The nerves lie free, each within the lumen of the corresponding artery. The anterior portion of the periganglionic sinus is continued in the form of a cylindrical sinus which, for distinction, may be termed the *peripharyngeal sinus*. The pheripharyngeal sinus encloses the oesophagus and pharynx and also the nerve trunks which supply the parts of the capitulum, and extends in the basis capituli almost to the buccal cavity. Near its proximal end, a pair of arterial trunks arise from its dorsal wall and pass directly upwards to enter the posterior extremities of the chelicerae (see Text-fig. 5). Like the pedal trunks, these *cheliceral arteries* contain within their lumina the cheliceral nerves. At its distal extremity the periganglionic sinus apparently

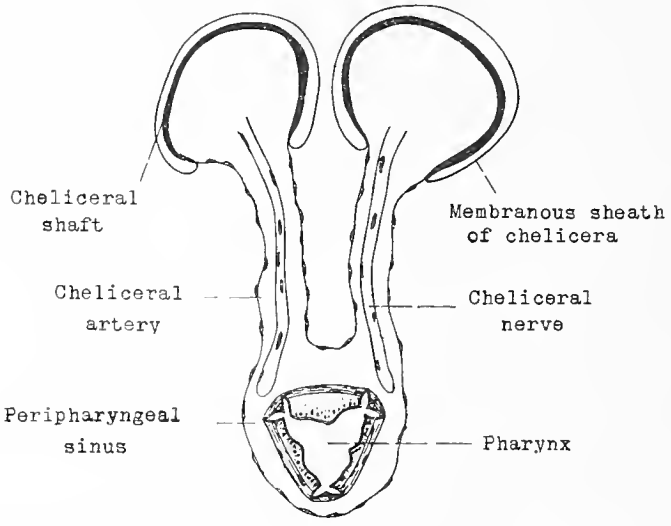


Fig. 5. *Argas persicus* ♂. Transverse section of the peripharyngeal sinus at the place of origin of the cheliceral arteries. ×150 diam.

divides into right and left branches, each of which enters the base of the corresponding palp. We have not been able to define a *postero-median* arterial trunk, but at the point where the splanchnic nerves issue from the brain, the periganglionic sinus in all probability gives rise to such a vessel.

The walls of the sinuses and vessels are formed of an exceedingly thin membrane which presents the appearance of a flattened epithelium.

From the foregoing description it is seen that the central and peripheral nervous systems are continually laved by the blood-stream which is directly propelled by the contractions of the heart, and further, the

appendages and pharyngeal muscles receive their blood supply from the same source. The blood finds its way back to the heart through the lacunar spaces between the organs of the body. The coelomic space immediately round the heart may be looked upon as a pericardial sinus, though no enclosing membrane, comparable with that of the above-described sinuses, can be identified. Active circulation of the blood is brought about in the middle and hinder parts of the body by the contractions of the dorso-ventral body-muscles.

The blood of the tick is a colourless fluid, consisting of a clear plasma in which are suspended large nucleated amoeboid corpuscles (see Pl. XVII, fig. 2, *bl. cp.*) which are sufficiently numerous to make a drop of blood such as issues from a puncture in the body-wall of the living animal appear distinctly turbid.

## The respiratory system.

Part I, Plate III, fig. 6; Part II, Plates XIV and XVI.
Text-figures 6 and 7.

The respiratory organs of *A. persicus* resemble those of the *Ixodidae*, in that they consist of a pair of spiracles and a highly developed system of tracheal tubules.

The literature on the subject having been reviewed in detail by Nordenskiöld[1], Bonnet[2] and Samson[3], it is unnecessary in this paper to repeat their remarks.

The position and external appearance of the spiracles received consideration in connection with the anatomy of the external parts of this tick (see Part I, pp. 32–33).

In 1908, Nuttall, Cooper and one of the present authors (L. E. R.) made a special study of the spiracle of an Ixodid tick (*Haemaphysalis punctata*)[4], and a comparison of the structures then described, with the corresponding parts of the spiracle of *A. persicus*, makes an interesting study; but before making such a comparison, it would, perhaps, be better to proceed first with a description of the internal structure of the spiracle of the latter species.

Externally, the spiracle appears as a small, nearly circular, salient boss which protrudes from the ventral body-wall. Its surface is divided

[1] Nordenskiöld, E. (1909), p. 456.
[2] Bonnet, A. (1907), pp. 44–50.
[3] Samson, K. (1909 *a*), pp. 205–209.
[4] Nuttall, G. H. F., Cooper, W. F., and Robinson, L. E. (xii. 1908).

by a deep cleft into two unequal parts:—(*a*) a smaller narrowly crescentic area which forms the anterior and the greater part of the lateral margin, and (*b*) a larger postero-internal area which fills in the remainder of the rounded contour of the spiracle (see Part I, Pl. III, fig. 6). As previously stated, the anterior crescentic area is perforated by innumerable fine pores, while the larger postero-internal portion of the spiracle is formed of stout cuticle which differs but little in appearance from those parts of the general cuticular investment of the body from which the scutellae are absent (see Part I, p. 29).

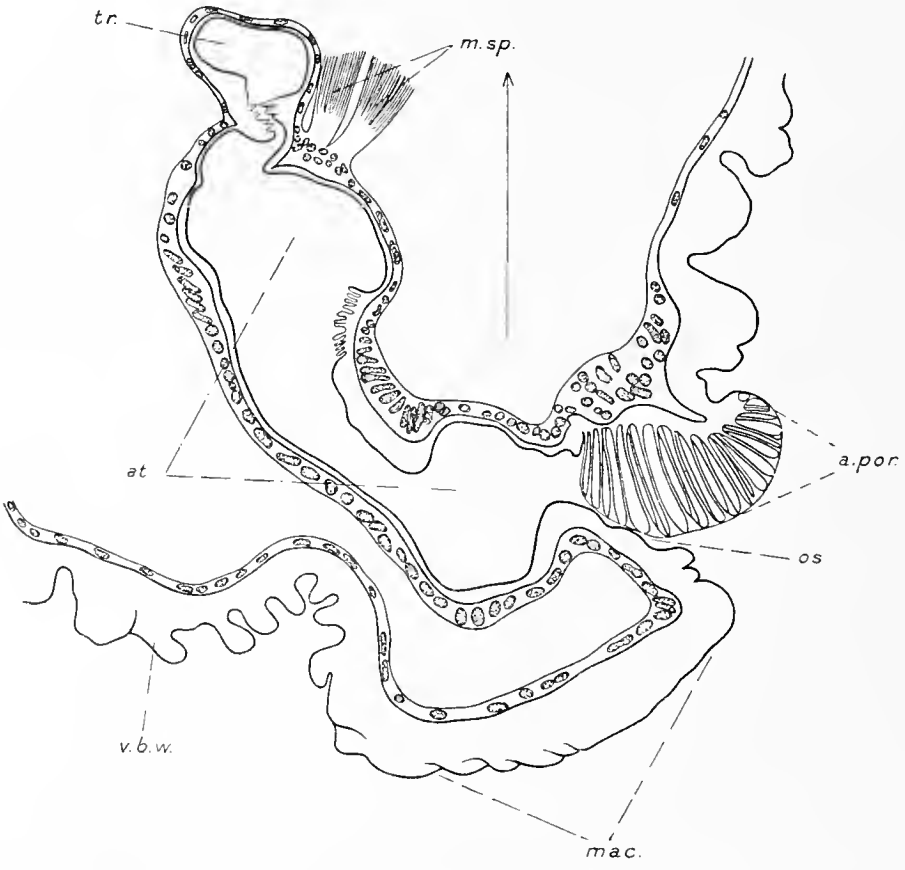


Fig. 6. *Argas persicus* ♂. Transverse section through the spiracle and atrium. The section is orientated in its natural position. ×380 diam.

Text-figure 6 represents a transverse section through the spiracle and immediately underlying parts. The lower portion of the figure shows the postero-internal imperforate area (*mac.*), which is separated from the perforated crescentic portion (*a. por.*) by the above-mentioned deep cleft (*os.*). In the upper portion of the left half of the figure, a hollow chamber of irregular form—the *atrium* (*at.*)—is seen to project

within the body-cavity and to communicate with the exterior through the cleft. The section through the crescentic plate shows it to consist of a deep layer of chitinous cuticle to which the hypodermis is apposed, and from the outer surface of which large numbers of slender chitinous processes extend to the surface of the crescentic plate where they appear to fuse together by their expanded extremities to form a continuous outer plane of thin chitin. Close examination shows, however, that between the outer extremities of these slender processes, an occasional hiatus appears in the section, which occurs where the minute pores, with which the surface of the crescentic plate is scattered, are cut across.

If, now, these structures are compared with those shown in a section through the spiracular plate of *Haemaphysalis punctata*, it is evident that the postero-internal area (*mac.*) corresponds to what was described in the paper cited, as the *macula*, which, in all the Ixodid ticks, occupies an isolated position in or near the middle of the spiracular plate, being completely cut off from the general body cuticle by the *area porosa* (*a. por.*). The latter is represented in a reduced degree by the perforate crescentic area of the spiracle of *A. persicus*, in which case it falls far short of completely surrounding the macula. The slender chitinous processes are homologous with the *pedicels* of *H. punctata*. The pyriform spaces and the internal pores of the latter species are absent in *A. persicus*. The pores of the area porosa establish a communication between the *interpedicellar* air spaces and the exterior, and the interpedicellar air spaces are continuous with the cavity of the atrium.

The atrium is a somewhat elongated cavity the lumen of which is contracted about its middle. It communicates with the exterior through the ostium and also, by way of the interpedicellar air spaces, through the pores of the area porosa. Its walls are composed of two layers—an inner *hypodermis* and an outer chitinous *cuticle*—both of which are continuous with the general integument of the body. The hypodermal layer possesses larger and more numerous nuclei than that of the general integument, but is otherwise identical in structure. The constriction of the lumen of the atrium, mentioned above, is brought about by the fact that a limited portion of the atrial wall, the cuticle of which is thicker than the rest, bulges out into the cavity; this projection apparently serving to close the atrium by being squeezed against the opposite wall of the cavity, an operation which is possibly effected by the contraction of the dorso-ventral body muscles. Immediately internal to this structure, the cuticular lining of the atrium shows a series of fine parallel ridges (see Text-fig. 6) which, though they do not receive

outgrowths of the hypodermis, suggest a rudimentary "book-gill" formation. A small muscle which originates on the inner surface of the dorsal body-wall is inserted into the wall of the atrium near its base. This muscle appears to act as a dilator of the atrium, and is evidently homologous with the *columellar muscle* of the spiracle of *Haemaphysalis punctata*.

The main tracheal trunks (see Text-fig. 7), usually five in number, open from the lower or innermost extremity of the atrium; and almost from their point of origin they diverge, each to follow its respective course in the body of the tick. The distribution of the five trunks is as follows:

(1) A large *anterior trunk* (*tr. ant.*) runs directly forwards along the ventral body-wall and passes beneath the salivary gland where it

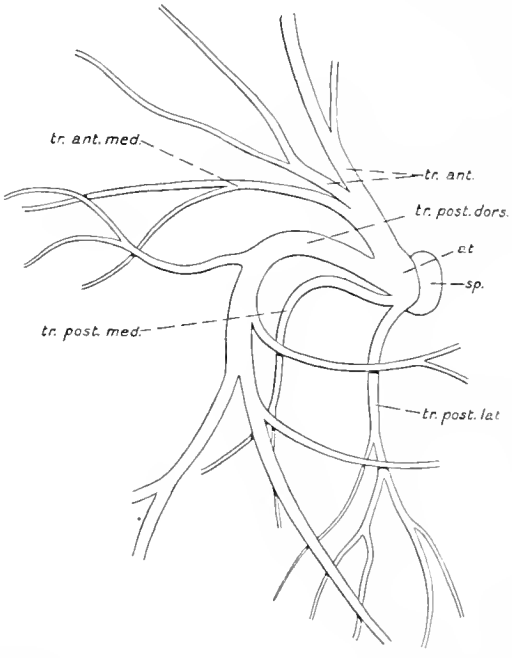


Fig. 7. *Argas persicus* ♀. Diagram showing the mode of origin and the disposition of the principal tracheal trunks. ×30 diam.

divides into a large number of branches, some of which run towards the middle line of the body where they form an anastomosis beneath the brain with corresponding branches from the opposite side of the body. Other branches are distributed to the oesophagus and pharynx, the capitulum and its appendages, the first pair of legs and the anterior part of the body generally.

(2) An *antero-median trunk* (*tr. ant. med.*) which supplies branches to the second, third and fourth legs and the coxal muscles, other

branches running to the middle line of the ventral body-wall, where they anastomose freely with their partners of the opposite side.

(3) A large *postero-dorsal trunk* (*tr. post. dors.*) which curves upwards through the great notch between the antero-lateral and postero-lateral lobes of the stomach and then continues its course over the dorsal surface of the postero-lateral lobe. At the point where this trunk appears on the dorsal surface of the gut, a short branch arises which runs inwards and forms an anastomosis which surrounds the heart. The remainder of the branches of the postero-dorsal trunk are distributed over the whole postero-dorsal region of the body.

(4) A *postero-median trunk* (*tr. post. med.*) runs towards the median line and then backwards, to furnish branches to the inner half of the postero-ventral region of the body.

(5) A *postero-lateral trunk* (*tr. post. lat.*) leads directly backwards from the atrium and divides into branches which ramify in the outer half of the postero-ventral part of the body.

Variations from the courses described above are generally due to the fact that the smaller trunks such as the *antero-median* and the *postero-median*, instead of proceeding directly from the atrium, arise as branches from the proximal portions of the large *anterior* and *postero-dorsal* trunks, respectively. Such a case appears in the figure (Text-fig. 7), in which the *antero-median* trunk is seen to take its origin from the *anterior* trunk.

The ultimate divisions of the tracheae are exceedingly fine, and as they ramify over the surfaces of the organs they may be seen to penetrate between the cells. In *I. ricinus* Nordenskiöld[1] has observed that the terminal twigs of the tracheae actually enter the bodies of the cells; he specifically mentions the secretory cells of the hypodermal glands, the nerve cells and, particularly, the contractile substance of the body muscles.

The histological structure of the tracheae of the *Ixodoidea* has been described by most of those who have worked on the anatomy of ticks. Though there appears to be some doubt as to whether they are homogenetic with the tracheae of the *Antennata*, they are apparently identical in structure. Each trachea possesses an inner cuticular sheath which is spirally thickened, and an outer epithelium of flattened cells containing a finely granular cytoplasm and flattened nuclei, the same structure extending from the largest tracheal trunks to the finest ultimate twigs.

[1] Nordenskiöld, E. (1909), pp. 457–458, and Pl. 30, figs. 4 and 5.

## The Excretory System.

In addition to the previously described Malpighian tubules (p. 238) a pair of *coxal glands* is developed in *Argas persicus*, the function of which is probably concerned with the excretion of waste products. The subject is one which requires further investigation before any definite opinion can be expressed.

### The Coxal Glands.

Plate XVII, fig. 9; Text-fig. 8.

The coxal glands, which were first observed in *Ornithodorus* by Christophers, and subsequently described in *O. moubata* by von Künssberg[1], appear in *A. persicus* as a pair of very small glandular masses which lie immediately above the second pair of coxae. They are to be

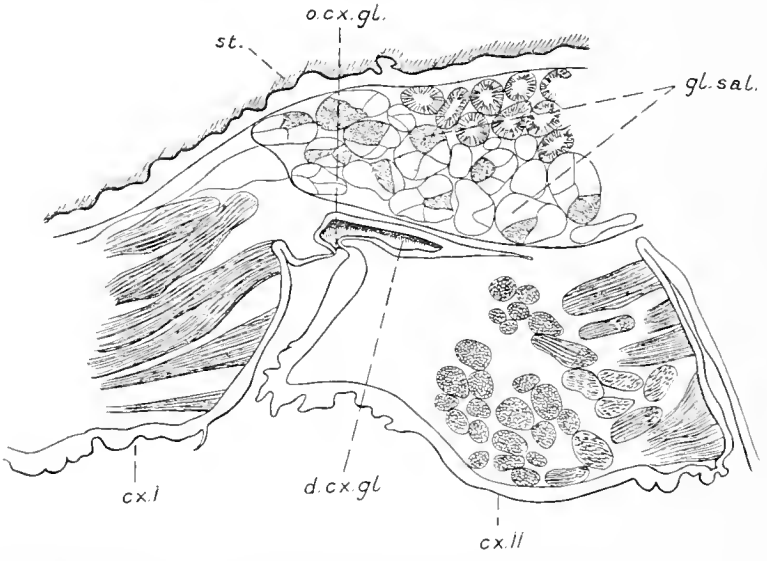


Fig. 8. *Argas persicus* ♀. Longitudinal section passing through coxae i and ii, showing the duct of the coxal gland and its external opening in the coxal interspace. × 100 diam.

recognised with difficulty in dissections of the entire animal, but are readily seen in sections (see Pl. XVI, fig. 2, *o. ex. gl.*). Each gland consists of a small number of relatively large alveoli which are each separately connected with the main duct by a short efferent duct. In some respects the alveoli resemble the *Pyramidenzellen* of the salivary glands (see p. 224). The nuclei are very large and kidney-shaped; they are rich in chromatin which is disposed in coarse flecks, and always contain one or more large

[1] Künssberg, K. von (1911).

nucleoli. The protoplasm is finely granular, but exhibits a distinct reticulation and shows no subdivision into individual cells. The lumen of each alveolus appears to be formed by a number of intercommunicating lacunae which occupy the centre of the alveolus. The ducts of the coxal glands exhibit precisely the same structure as the salivary glands. They are lined by a chitinous intima which is spirally thickened, and the outer epithelial sheath is identical in its appearance with that of the corresponding structure in the salivary glands. The main duct of the gland after receiving the efferent ducts of the alveoli, runs inwards and forwards to the interspace between the first and second coxae where it opens as has been described in Part I of this paper (*Parasitology* VI. pp. 33–34). Associated with the main duct of the coxal gland is a small saccular diverticulum with thick walls composed of a single layer of cylindrical cells. The diverticulum opens into the duct almost at the termination of the latter at the external orifice, but what its function may be, we are at a loss to explain.

The function of the coxal glands is still uncertain. In his work on the anatomy of *Ornithodorus savignyi*, Christophers[1] gives a short account of the extrusion of fluid from the coxal glands (p. 10), his remarks being based on information derived from Major Donovan, I.M.S. The fluid is discharged while the operation of feeding is in progress, and Christophers states that the secretion is slightly alkaline and that it prevents the coagulation of blood. In the same memoir (p. 45) he briefly describes the structure and relations of these glands in *O. savignyi*, from which it would appear that they are very similar to those of *Argas persicus*.

Leishman[2] appears to have been the first to demonstrate that the coxal fluid of *O. moubata* prevents the coagulation of the blood. Künssberg[3] cites Christophers' observations, and proved by actual experiment, that the secretion of the coxal glands of *O. moubata* inhibited the coagulation of the blood of dogs and rabbits. She concluded that the function of the glands was the secretion of an anti-coagulin, and that it is analogous with the *Funduszellen* of the salivary glands of *Ixodes ricinus*.

If this conception of the function is the correct one, the fluid discharged during the operation of feeding into the space between the ventral surface of the tick and the body of the host, passes by capillary attraction, presumably, to the wound, where it mingles with the issuing blood, the mixed fluids then being ingested simultaneously by the tick.

[1] Christophers, S. R. (1906), pp. 10 and 45.

[2] Cited by Nuttall and Strickland (1908), p. 310.

[3] Künssberg, K. von (1911).

In *Argas persicus,* however, the secretion only appears *after* engorgement, large droplets of clear colourless fluid being discharged within a few seconds or minutes of the withdrawal of the proboscis of the tick from the wound.

The explanation offered by Nuttall[1] is, in all probability, the true one, viz. "that the object of this secretion appears to be to permit the tick to concentrate the essential food-constituents of the blood in its alimentary canal." The presence of an anticoagulin in the secretion is doubtless explained by the fact that the salivary secretion, which is ingested with the blood, undoubtedly contains such a principle, and any excess would remain in solution in the non-nutrient watery constituents of the blood.

It is most remarkable that these two tiny glands should be capable of excreting such a large quantity of fluid in so short a time. In an experiment which was made by W. F. Cooper and one of us (L. E. R.), a fasting female, sent from Rhodesia, was allowed to gorge to repletion on a fowl. Immediately it detached itself, it was removed to a small glass capsule, the weight of which had been accurately determined, and the capsule containing the tick was again weighed, the difference between the two weighings giving the weight of the gorged tick. After a minute or two, the tick was seen to discharge, quite suddenly, a large drop of secretion from the coxal glands, and simultaneously underwent an apparent diminution in size. After draining as much as possible of the adherent fluid from the surface of the tick's body and finally drying it with filter-paper, the tick was again weighed. The original weight of the gorged tick was 0·0385 gm.; the weight of the tick after the extrusion of the coxal gland secretion was 0·0245 gm.; the difference between these figures represents the weight of the secretion, viz. 0·0140 gm.—36·6 % of the original weight of the tick! In all the cases observed, extrusion of the coxal gland secretion occurred within five minutes of the detachment from the host.

Coxal glands, the function of which is undoubtedly concerned with the excretion of nitrogenous waste products, occur in many *Arachnida,* but in all known examples the openings of the excretory ducts are situated on the fifth pair of appendages, which correspond to the third pair of legs in the tick. The subject is one which requires further investigation to clear up the problem.

[1] Nuttall, G. H. F. (VII. 1908), p. 14.

## EXPLANATION OF PLATES XIV TO XVII.

### PLATE XIV. *Argas persicus.*

Dissection of young female, from the dorsal surface. The left-hand half of the figure represents the internal organs as they appear after the removal of the dorsal integument. On the right-hand side, the stomach and alimentary coeca have been removed to display the more deeply-placed structures. Magn. 25 diam.

### PLATE XV. *Argas persicus.*

Median longitudinal section of the body of the male, anterior to the anus (slightly schematised). Magn. 40 diam.

### PLATE XVI. *Argas persicus.*

A series of six transverse sections of the body of the male. Magn. 45 diam.

Fig. 1. Transverse section at the level of the middle of the basis capituli.
Fig. 2. Transverse section at the level of the genital aperture.
Fig. 3. Transverse section at the level of the anterior bifurcation of the stomach.
Fig. 4. Transverse section at the level of the junction of the oesophagus with the stomach.
Fig. 5. Transverse section at the level of the spiracles.
Fig. 6. Transverse section at the level of the anus.

### PLATE XVII. *Argas persicus.*

Fig. 1. Female; vertical section of a portion of the integument from the ventro-lateral part of the body. Carnoy's Fluid, Thionin-Eosin. (Owing to an accidental precipitation in the tissues, the canaliculi, which run from the inner surface towards the outer surface of the cuticle, are very beautifully demonstrated in this preparation.) × 600 diam.

Fig. 2. Female; vertical section of a portion of the stomach wall. Carnoy's Fluid, Thionin-Eosin. × 600 diam.

Fig. 3. Male; portion of wall of stomach, flat preparation for demonstration of muscle-fibres in outer sheath. (The fresh tissue was spread out flat on a glass slip; fixed with Carnoy's Fluid, and after removal of a considerable portion of the epidermis by the gentle application of a fine camel-hair brush, the preparation was stained with Ehrlich's "Triacid" mixture.) × 180 diam.

Fig. 4. Male; transverse section passing through anal canal and anus. Carnoy's Fluid, Thionin-Eosin. × 350 diam.

Fig. 5. Female; salivary gland, alveolus of *first type* (granule-secreting). Flemming's Fluid, Heidenhain's Iron-alum-Haematoxylin. × 500 diam.

Fig. 6. Female; salivary gland, alveolus of *second type* (the *Pyramidenzellen* of Samson). Preparation as in previous example. × 500 diam.

Fig. 7. Male; transverse section of Malpighian tubule. Carnoy's Fluid, Thionin-Eosin. × 320 diam.

Fig. 8. Female; longitudinal section through heart, a short distance from the median line. Carnoy's Fluid, Ehrlich's Haematoxylin-Eosin. × 350 diam.

Fig. 9. Female; two alveoli and portion of duct of coxal gland. Carnoy's Fluid, Thionin-Eosin. × 600 diam.

## INDEX TO LETTERING ON PLATES XIV TO XVII.

| | |
|---|---|
| *an.* | anus. |
| *an. ap.* | anal aperture. |
| *an. can.* | anal canal. |
| *an. m.* | anal annulus. |
| *an. v.* | anal valve. |
| *ao.* | aorta. |
| *at.* | atrium. |
| *b. c.* | basis capituli. |
| *bl. cp.* | blood corpuscles. |
| *br.* | brain. |
| *buc. can.* | buccal canal. |
| *buc. cav.* | buccal cavity. |
| *cam.* | camerostome. |
| *cam. f.* | camerostomal fold. |
| *cc. al.* | alimentary coeca. |
| *c. g. com.* | common genital canal. |
| *c. gu.* | guanin concretions. |
| *ch.* | chelicerae. |
| *cx. i–iv.* | coxae i–iv. |
| *cx. f.* | coxal fold. |
| *d. ch.* | digit of chelicera. |
| *d. cx. gl.* | duct of coxal gland. |
| *d. ov.* | oviduct. |
| *d. sal.* | salivary duct. |
| *end.* | endosternite. |
| *f. c.* | capitular foramen. |
| *gen. ap.* | genital aperture. |
| *Gen. org.* | Gené's organ. |
| *gl. Gen. org.* | glandular portion of Gené's organ. |
| *gl. sal.* | salivary gland. |
| *gl. w. a.* | anterior lobes of white gland. |
| *gl. w. p.* | posterior lobes of white gland. |
| *h.* | hypostome. |
| *ht.* | heart. |
| *l. 2–4* | legs 2–4. |
| *l. ht.* | lumen of heart. |
| *m. ab. cx. ii.* | abductor muscle of coxa ii. |
| *m. ab. p.* | abductor muscle of palp. |
| *m. ad. cx. iv.* | adductor muscle of coxa iv. |
| *m. an.* | anal muscles. |
| *m. c. ph.* | constrictor muscles of pharynx. |
| *m. d. c.* | depressor muscles of capitulum. |
| *m. d. ph.* | dilator muscles of pharynx. |
| *m. f. d.* | flexor muscle of digit of chelicera. |
| *m. gen.* | genital muscles. |
| *m. gen'.* | genito-dorsal muscles. |

| | |
|---|---|
| *m. ht.* ... ... | extrinsic muscles of heart. |
| *m. ht'.* ... ... | intrinsic muscles of heart. |
| *m. l. c.* ... ... | levator muscles of capitulum. |
| *m. l. int. cx. i and ii.* | lateral intercoxal muscles i and ii. |
| *m. mg.* ... ... | marginal dorso-ventral body-muscles. |
| *m. m. int. cx. i–iii.*... | mesial intercoxal muscles i–iii. |
| *m. p. a.* ... ... | postero-accessory dorso-ventral body-muscles. |
| *m. p. m.* ... ... | postero-median dorso-ventral body-muscles. |
| *m. r. ch.* ... ... | retractor muscles of chelicerae. |
| *m. st.* ... ... | muscular sheath in wall of stomach. |
| *m. sub, cx. i.* ... | inferior subcoxal muscles. |
| *n. l. i. and ii.* ... | nerves of leg 1 and 2. |
| *o. cx. gl.* ... ... | orifice of duct of coxal gland. |
| *oes.* ... ... | oesophagus. |
| *o. gl. w.* ... ... | orifices of the different lobes of the white gland. |
| *o. ph.* ... ... | pharyngeal orifice. |
| *os. ht.* ... ... | ostium of heart. |
| *ov.* ... ... | ovary. |
| *ph.* ... ... | pharynx. |
| *sal. gl.* ... ... | salivary gland. |
| *s. ch.* ... ... | outer sheath of chelicera. |
| *s. ch'.* ... ... | inner sheath of chelicera. |
| *s. cx. f.* ... ... | supracoxal fold. |
| *sp.* ... ... | spiracle. |
| *s. pg.* ... ... | perigangliouic sinus. |
| *s. r.* ... ... | rectal sac. |
| *st.* ... ... | stomach. |
| *sub. ch. p.* ... | subcheliceral plate. |
| *t. c.* ... ... | rectum. |
| *tes.* ... ... | testis. |
| *t. mpg.* ... ... | Malpighian tubule. |
| *tr.* ... ... | tracheae. |
| *tr'.* ... ... | tracheal anastomosis beneath central nervous system. |
| *ut.* ... ... | uterus. |
| *v. d.* ... ... | vas deferens. |
| *v. d. c.* ... ... | common canal of vasa deferentia. |
| *v. os. ht.* ... ... | valvular flap of ostium of heart. |

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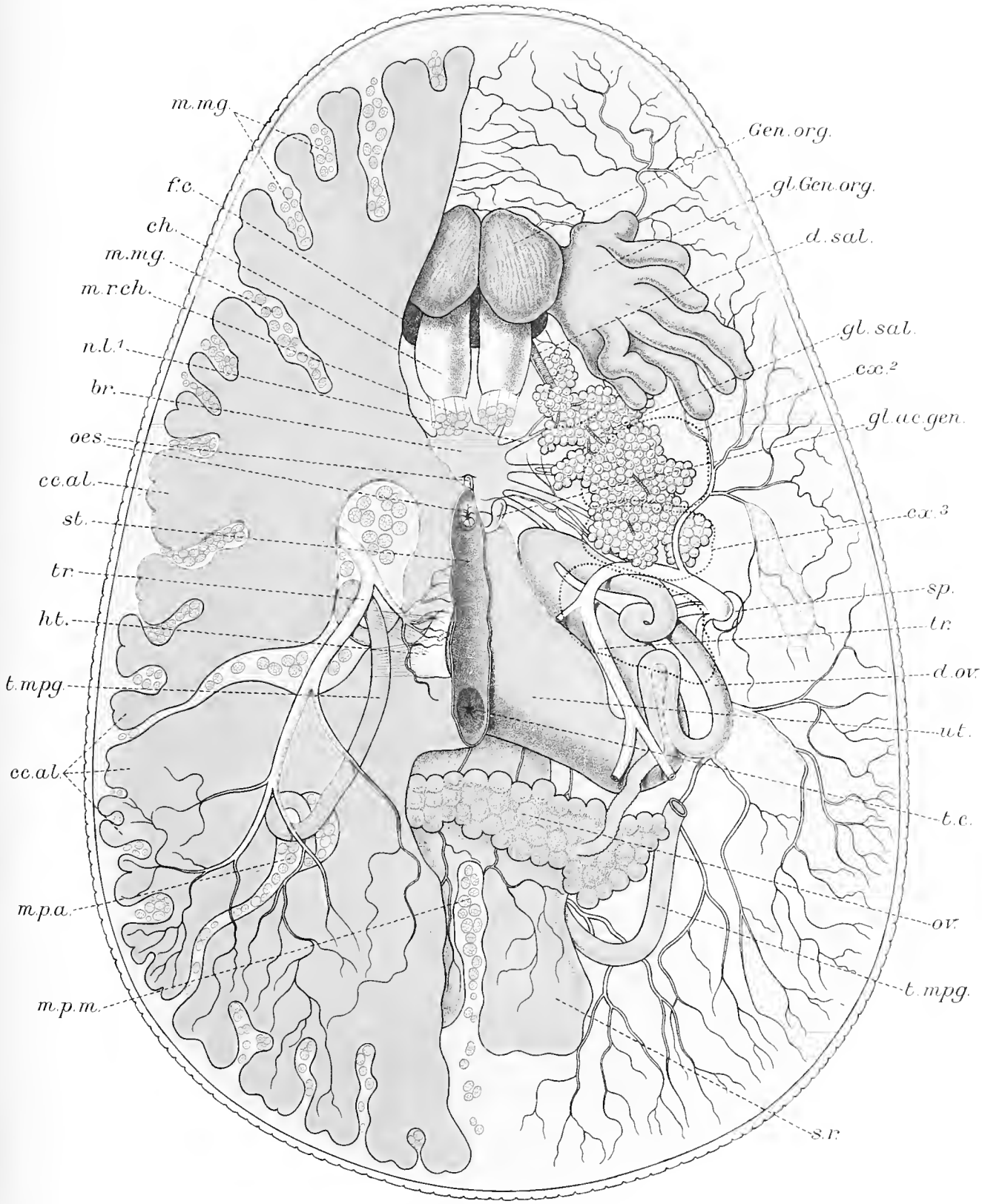
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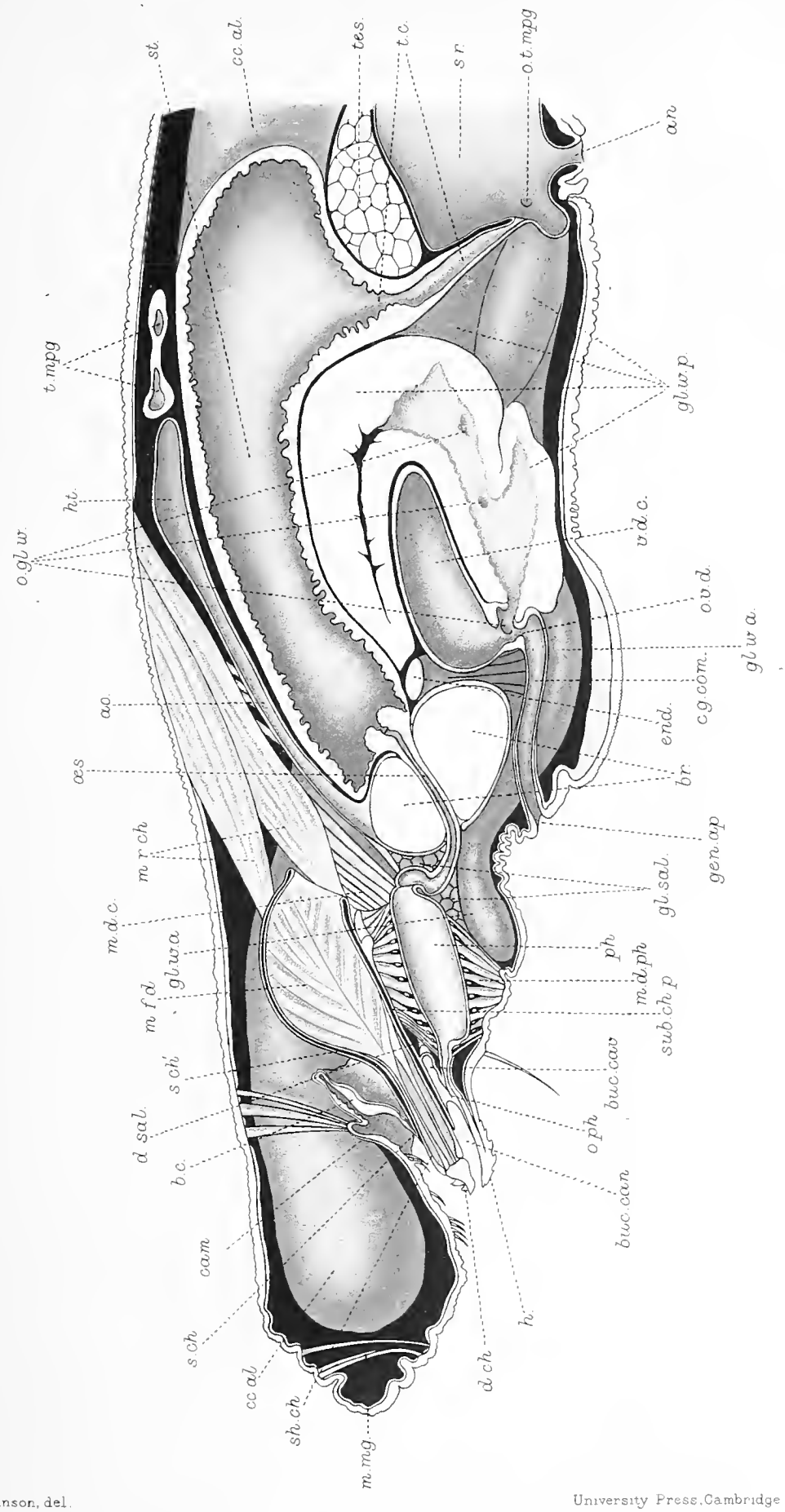


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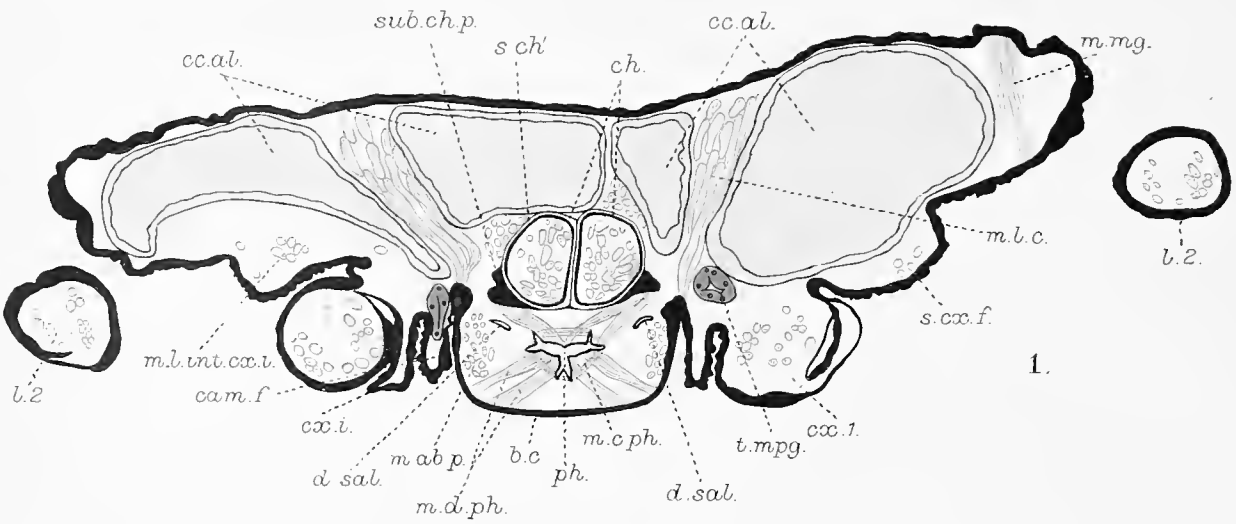


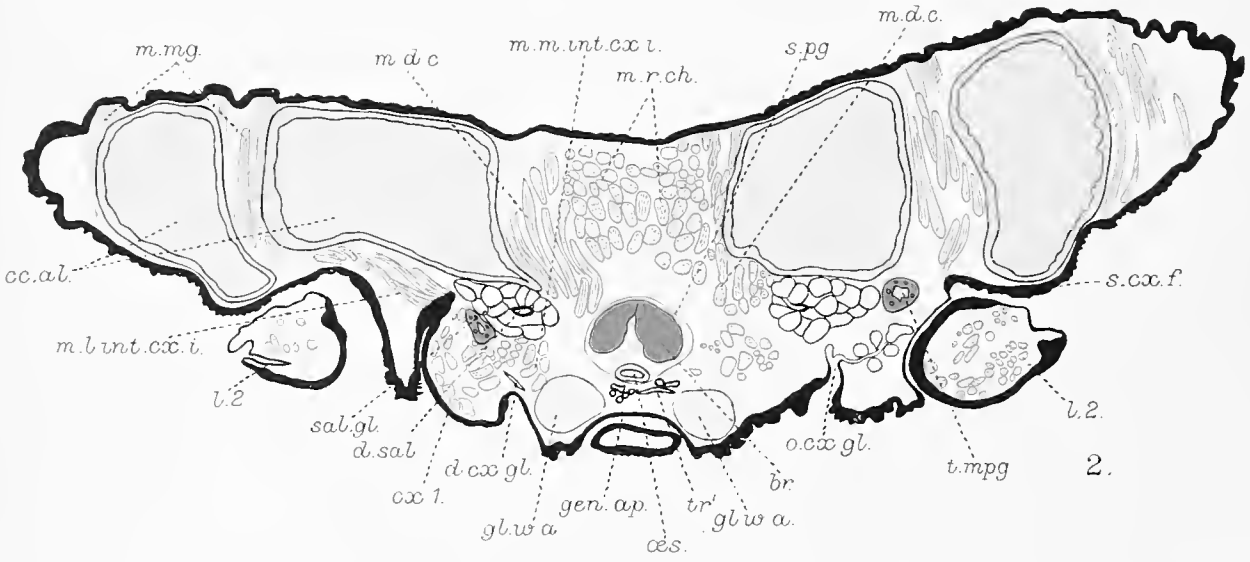


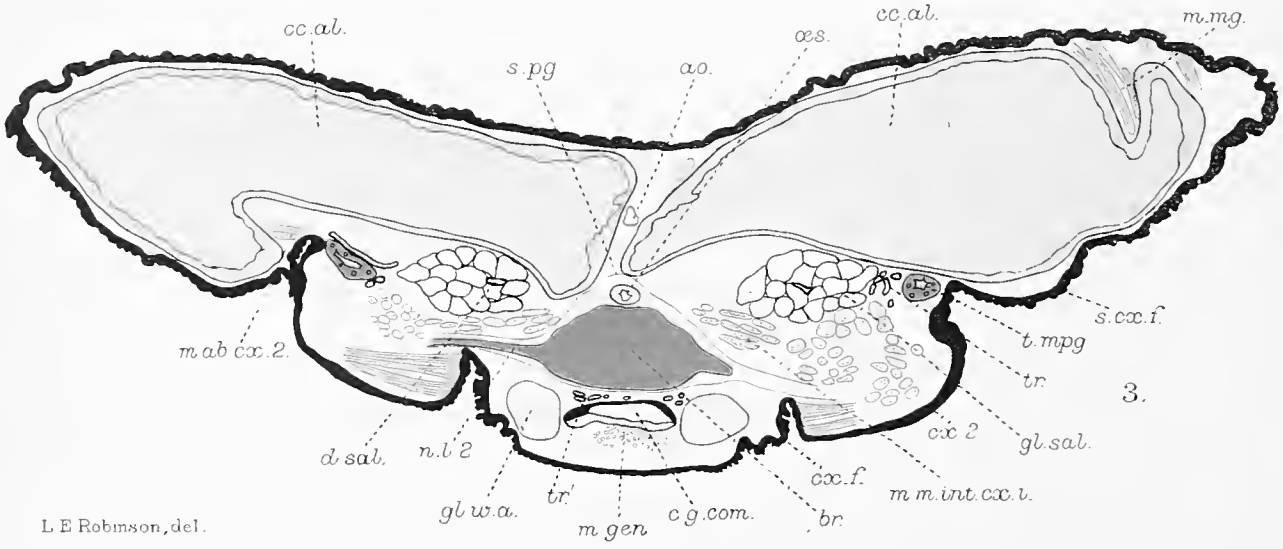


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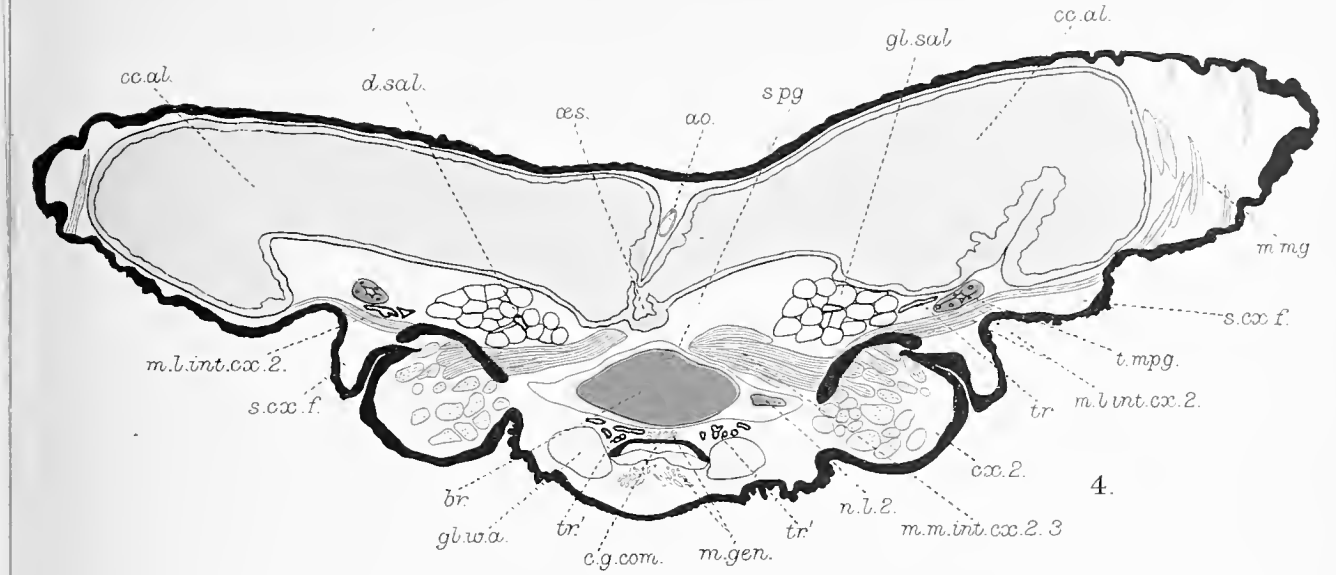


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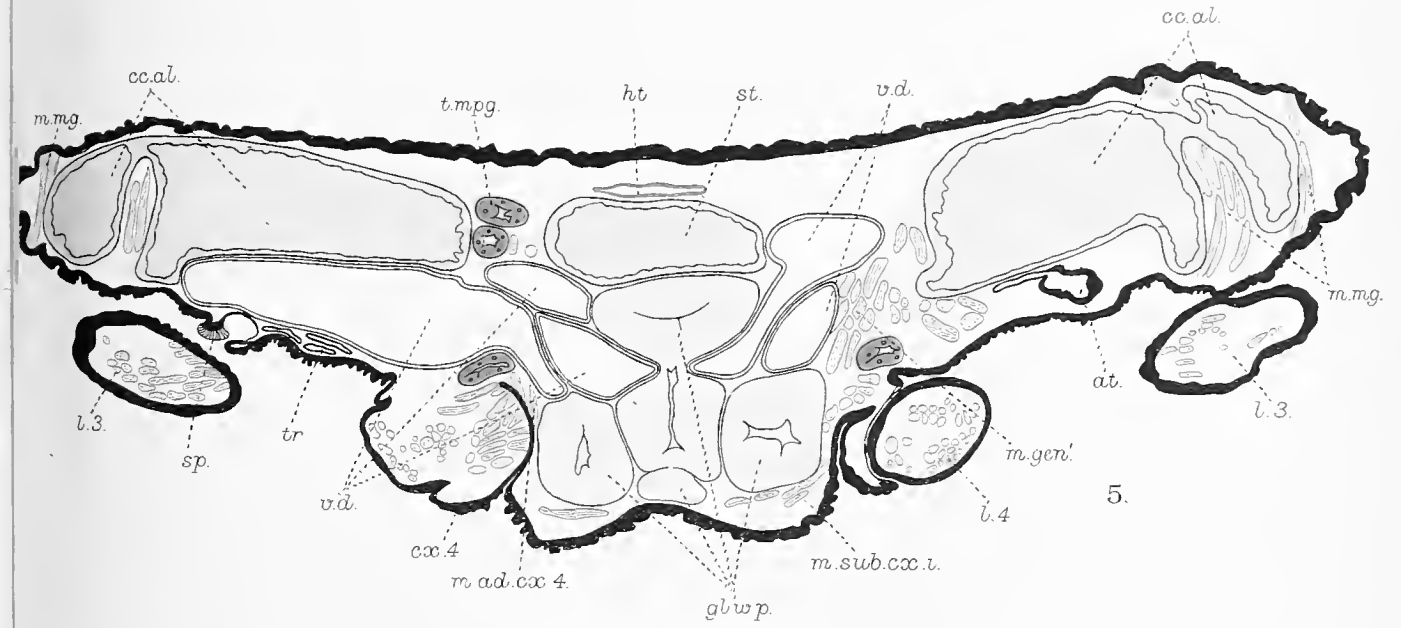


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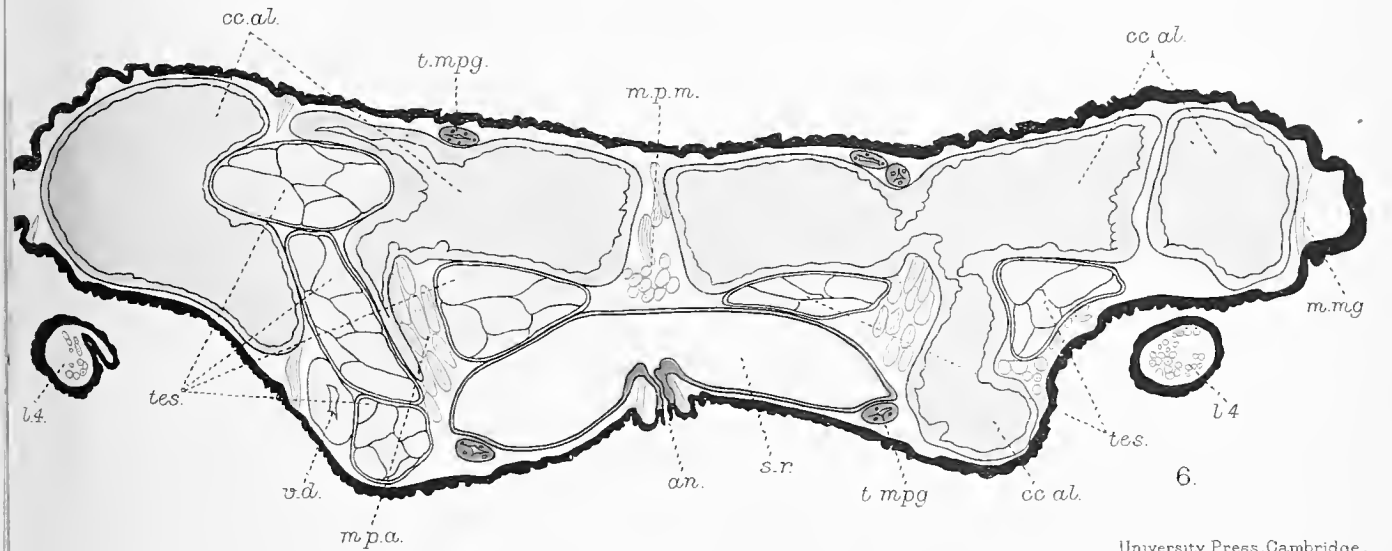


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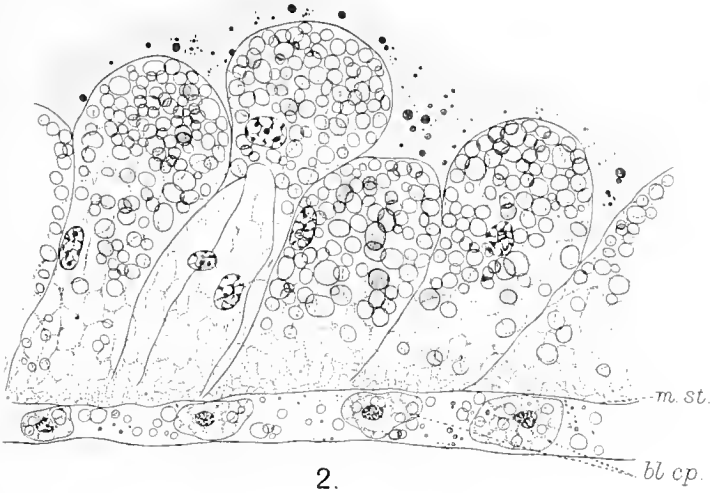
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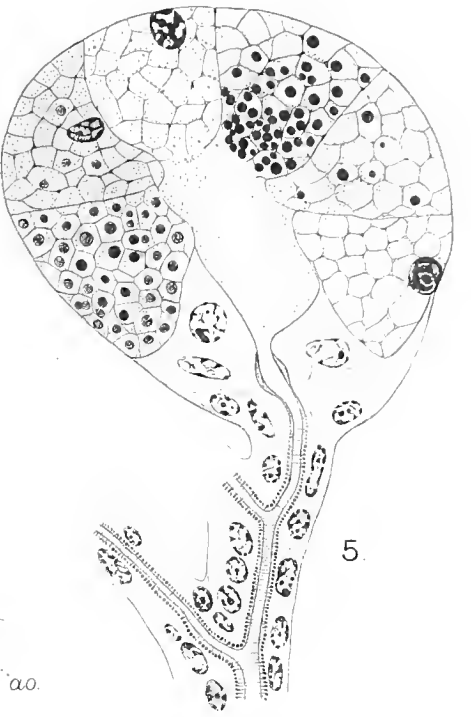

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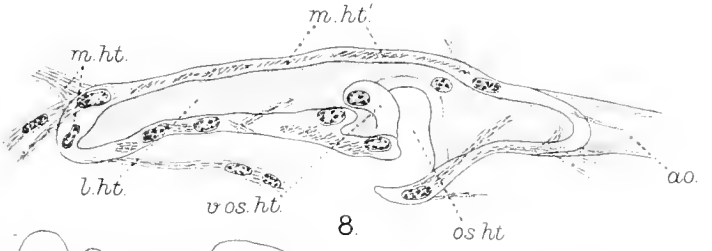
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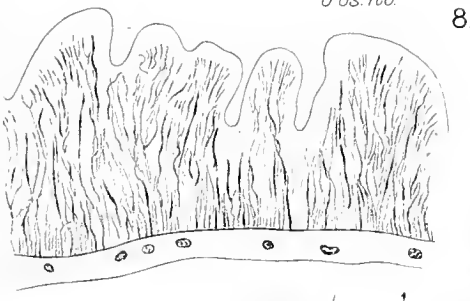

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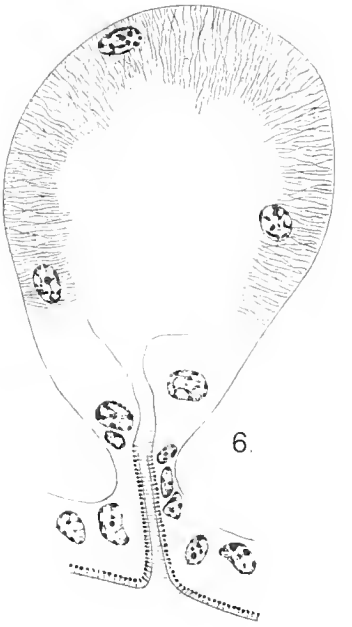

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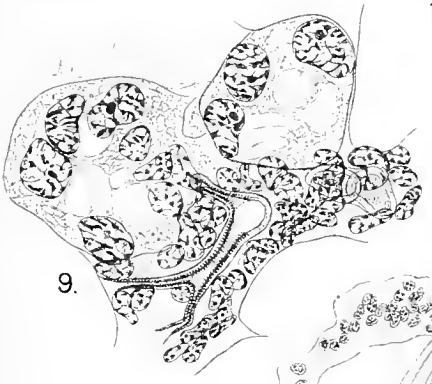

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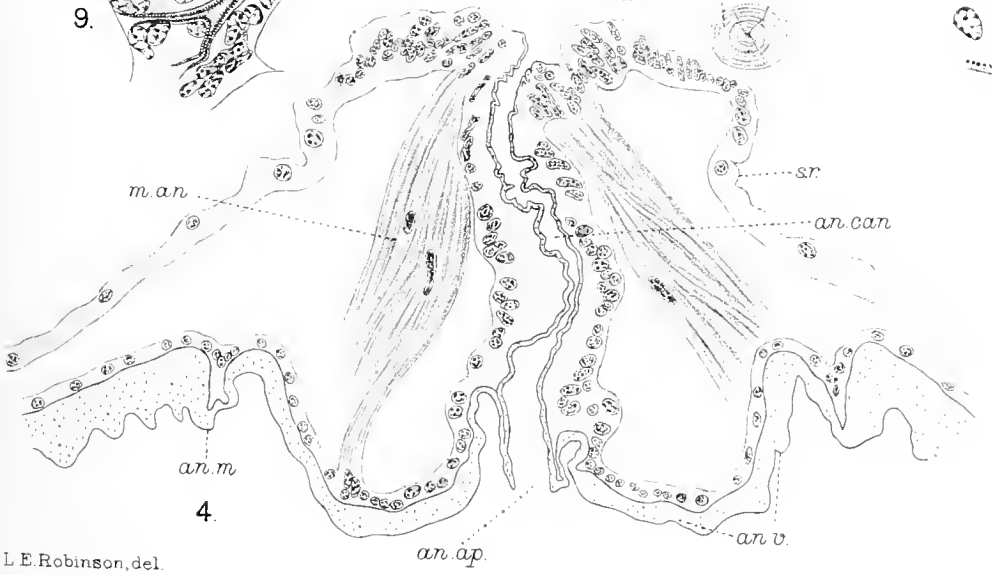
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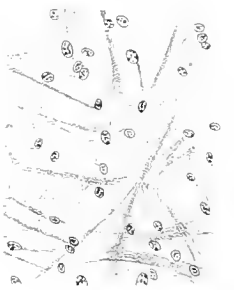

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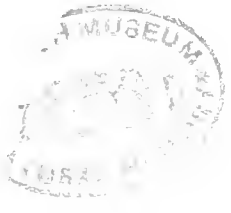

# THE NUCLEAR STRUCTURE AND THE SPORULATION OF *AGRIPPINA BONA* STRICKLAND.

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*Assistant to the Quick Professor of Biology.*

(*From the Quick Laboratory, Cambridge.*)

(With Plate XVIII and 8 Text-figures.)

## Introduction.

*Agrippina bona*, a gregarine parasitic in the gut of the larva of the rat-flea *Ceratophyllus fasciatus*, was first described by Strickland in 1912. His account of the sporulation of the gregarine was so curious that a more detailed investigation has been undertaken, which has given rise to the present paper. The work has been done in the Quick Laboratory, under Professor Nuttall, to whom I wish to express my thanks for the interest he has taken in my researches, and for his constant encouragement.

Throughout the winter, the results that were obtained, although they did not confirm Strickland's account, pointed without exception to the origin of the gamete nuclei from chromidia. In the latter part of May, and in June, every cyst fixed in the stages preceding gametogenesis has shown clearly phases of the mitotic divisions which in almost all gregarines give rise to the gamete nuclei. I can only conclude that either all my earlier preparations of these stages were made from abnormal cysts, or that the gamete nuclei may be formed in one of two ways: by chromidiation, or at the end of a series of mitoses. As to the degeneration hypothesis, I must state that the score or so of cysts that were kept in hanging drops in the winter proceeded without exception to form spores, and even in roughly-sealed preparations under the

coverslip the sporulation was only rarely interrupted, and then by plasmolysis, owing to gradual concentration of the salt solution in which the cysts were immersed.

In this paper, the process of gametogenesis more usual in gregarines will alone be dealt with. If opportunity arises in the winter, I hope to be able to discover if chromidiation really occurs in *Agrippina* as a normal process.

### *Previous work on* A. bona.

Strickland has observed and figured the external form of spore, sporozoite, trophozoite, sporont and cyst. He conceives the karyosome of the trophozoite to consist of a closely wound skein, which later forms one or more rods or bands of chromatin. In the nucleus of the trophozoite and of the sporont, he finds spherical structures which he calls "polar bodies," which do not take up the nuclear stain. As will be seen later, to call these bodies achromatic is rather to misrepresent their staining reactions.

It is, however, Strickland's description of what occurs in the cyst which is most remarkable, and passages in his paper referring to the nucleus and to the spores are here quoted :—

".....the following nuclear changes can be made out. At first, while the two sporonts forming the cyst are still distinct, the nucleus of each half can be readily seen, but later, when the partition has disappeared, the nucleus cannot be seen under any circumstances. However, while it can be made out, it is seen to grow enormously in size, and its ultimate disappearance may be due to its having become commensurate with the cell. Meanwhile it is invested with a very definite nuclear membrane.

"The most striking change that has occurred in it is the loss of all its basic staining substance or chromatin, which cannot be seen in any form. The band-like riband of chromatin seen in the sporont has disappeared. The spherical bodies occurring in the nucleus of the sporont are now very numerous; the nucleus contains many of them irregularly scattered through the 'karyolymph,' and they may vary considerably in size.

"The details of the differentiation of the endoplasm into spores are very difficult to make out either in the living parasites or in stained preparations. Clear areas gradually loom up through the granular protoplasm, which is finally all used up, while the clear areas become

more definite and finally resolve themselves into the highly refringent spores."

The remarkable nature of the above account has not escaped criticism. Chatton (1912) appends to a summary of Strickland's paper the following note:—

"Nous connaissons cette grégarine. Disons dès maintenant qu'elle effectue sa sporogénèse suivant le mode normal: conjoints non fusionnés, formant, par perlage, des gamètes à peine inégaux, peu mobiles, qui copulent. Les sporocystes, d'abord périphériques, finissent par être enfermés dans le reliquat, faiblement amiboïde. La déhiscence est quelconque. La place de cette grégarine nous paraît dans la famille des Actinocephalides."

How thoroughly Chatton's note is justified will be seen in the following part of this paper.

## *The Trophozoite.*

In Text-fig. 1 is shown a stage in the transition between the sporozoite and the trophozoite. The gregarine figured is not yet segmented into protomerite and deutomerite, but is already provided with a rudimentary epimerite.

Fig. 1.

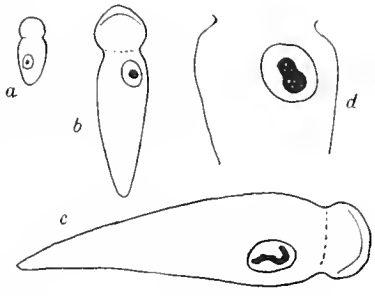


Fig. 2.

Fig. 1. Young trophozoite, showing rudimentary epimerite, but no differentiation into protomerite and deutomerite. Nucleus and faint striations visible. (From life.)

Fig. 2. Trophozoites from life. (*a*) Single karyosome in nucleus. (*b*) Refringent granules in karyosome. (*c*) Chromatin in band. (*d*) Irregular karyosome with refringent granules. *a*, *b*, *c* on same scale, *d* more highly magnified.

Neither Strickland nor I have found an intracellular stage in *Agrippina bona.*

A full account of the external morphology of the trophozoite is given by Strickland, and we shall here concern ourselves with the nuclear structure.

In young individuals the nucleus consists of a definite membrane containing karyolymph, in which occurs a fairly large karyosome. The karyosome may contain refringent granules which are visible *in vivo* (Text-fig. 2, *b*, *d*). I find no evidence that the karyosome is really a closely wound skein, as Strickland describes. (Pl. XVIII, fig. 1.)

In the nuclei of the sporonts, the place of the karyosome is usually taken by a bent, irregular rod or band, in which most of the chromatin is concentrated (Text-fig. 2, *c*). In nuclei of this stage there are usually present two (sometimes more) spherical bodies, called by Strickland "polar bodies," from their position. In preparations stained with Heidenhain's iron haematoxylin, these spherules stain, sometimes very intensely. The older sporonts, whilst still retaining the remains of the band of chromatin (now usually in fragments), have their most intensely staining material concentrated in the spherules, which appear much darker than the chromatin band. In fact, in measure as the chromatin band stains less and less darkly, the more intensely do the spherules take up the haematoxylin. A series illustrating this is given in Pl. XVIII, figs. 2–5.

With Giemsa's stain (wet method) the chromatin takes on a deep purple hue, whilst the spherules may stain a delicate rose pink, or may approximate to the chromatin coloration. It is difficult to obtain uniform results with this stain, but it seems probable that the spherules while yet achromatic stain pink, and later, when they contain chromatin, stain purple.

Altogether, it seems a fairly safe assumption that there is a gradual transference of chromatic material from the band to the spherules. The whole of the chromatin is, however, never transferred to the spherules; rods or granules always remain in the karyolymph, though they may stain but faintly.

### *The Cyst.*

Sections of very young cysts show clearly that association of the sporonts must occur "head to head," and not "tail to tail" as Strickland suggests.

This is evident from the persistence of the protomerites, which form, in longitudinal sections of young cysts, an equatorial body of lenticular shape, lying symmetrically about the boundary between the two associated individuals (Pl. XVIII, figs. 7, 8). This seems to be the stage at which staining material is most concentrated in the intranuclear spherules.

All trace of distinction between protomerite and deutomerite is soon lost, and the cyst is occupied by two masses of protoplasm separated by an equatorial septum. The spherules fade, but what the fate of their colourable material is, I am unable to say. They lose chromatin until they are very little darker than the karyolymph (Pl. XVIII, figs. 9, 10). They are usually present now to the number of six or more, though I have not been able to find out how the increase is effected.

Apparently concurrently with this—no connection is suggested—some process of chromidiation goes on, which results in the ectoplasm becoming faintly chromatic, and taking on a dark grey tone in Heidenhain preparations. This chromidiation has no connection with the formation of gamete nuclei, and a similar occurrence is not unknown in other gregarines (Pl. XVIII, figs. 9, 10).

At length the impoverished trophonucleus gives rise to the segmentation nucleus. Pl. XVIII, fig. 11 shows the old nucleus and the first spindle, the latter cut rather obliquely. The trophonucleus still contains a certain amount of chromatin, but no spherules are visible, and the nuclear membrane has disappeared. How the achromatic spindle is formed has not been ascertained, but from figs. 12 and 12 *a* it is fairly evident that the chromatin of the segmentation nucleus has been drawn directly from the trophonucleus. Attention is especially directed to the faint, grey-staining limb projecting from the trophonucleus to the spindle (Pl. XVIII, fig. 12).

The disorganised trophonucleus disappears, how quickly I am unable to say.

The further development within the cyst follows the normal type. By successive mitoses (figs. 13, 14) a large number of nuclei are formed, and these ultimately lie for the most part near the surface of the cytoplasm. I have not found any suggestion that reduction divisions occur immediately before gametogenesis, but the material is unfavourable for investigating this point.

*Formation of Gametes.*

This has been followed *in vivo.* Within the cyst-wall, the surface of the protoplasm becomes lobulated (Text-fig. 3). The line of separation between the individuals still persists. The lobulation becomes extreme, and at length the gametes arise from the surface as little ovoid protrusions (Text-fig. 4, *a*). They ultimately get cut off from the residuum (Text-fig. 4). There is a slight difference in size between the gametes arising from one sporont and those arising from the other in the cyst

(Text-fig. 4, *b*). The nuclei of both types of gamete appear to be of the same size, and are eccentrically placed (Text-fig. 4, *b*). The cytoplasm is granular. No trace of flagellation has been discovered in either gamete.

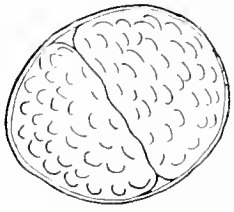

Fig. 3.

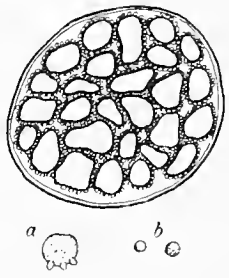


Fig. 4.

Fig. 3. Cyst showing lobulation of protoplasm.
Fig. 4. Cyst with gametes. (*a*) Formation of gametes. (*b*) The two sorts of gametes.

After the gametes have apparently been fully formed, the cyst may be watched for some hours without any movement being seen within it. It is a fair assumption that the gametes become mixed, so as to fuse each with a dissimilar one, but I do not know how the mixing process goes on. I have not seen the actual fusion of gametes, but Text-fig. 5, from a smear preparation of a cyst containing gametes, seems to show a stage in the fusion of two gametes.

Fig. 5.

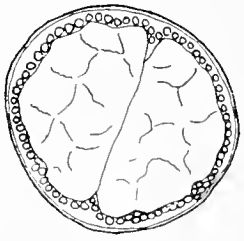

Fig. 6.

Fig. 5. Gametes from smear preparation (fixed). Possible fusion.
Fig. 6. Cyst with zygotes. (Optical section.)

*Spores.*

The zygotes are naturally rather larger than the gametes, and are at first disposed round the surface of the eystal residuum (Text-fig. 6). Then, possibly as a result of amoeboid motion of that residuum, as Chatton suggests, they become included within it (Text-fig. 7), and a wall of dense protoplasm appears inside the cyst-wall, whilst in the centre are numerous zygotes and irregular fragments of residual protoplasm (Text-fig. 8).

From this wall of dense protoplasm is formed the radially striated layer described by Strickland. I see no reason for supposing it to consist of tubes radially arranged.

The zygote remains uninucleate until it has embedded itself in a spore coat, within which the sporozoites are formed. In spite of repeated attempts, I have not been able to stain the contents of the maturing spores, and so can give no details of the nuclear divisions.

The dehiscence of the cyst is described by Strickland.

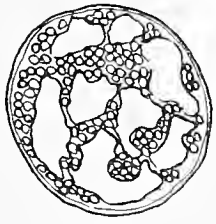

Fig. 7.

Fig. 8.

Fig. 7. Cyst with zygotes (later stage). Zygotes and residuum mixed.
Fig. 8. Cyst with zygotes (later stage). Dense protoplasm at periphery, zygotes and residuum in centre.

*Sexual Differentiation in* A. bona. As has already been described, there is a small difference between the gametes formed by the two individuals associated in a cyst. As both sorts of gametes seem to be non-motile, it is idle to speculate as to which corresponds to the spermatozoon and which to the egg.

There is also a difference between the sporonts, for in one series of sections, cut 6 $\mu$ thick and stained rather deeply, a very distinct difference in staining capacity was noticed between the associated sporonts, recalling that found by Brasil (1905) and by Léger and Duboscq (1909). Unfortunately I cannot say whether the more deeply tinted individual was to give rise to the larger or to the smaller gametes.

## *Taxonomy.*

There can be no question but that the gregarine under discussion represents a new genus. Strickland suggests a new family, the Agrippinidae, for its reception. To this Chatton takes exception, being of the opinion that *Agrippina* should be included in the Actinocephalidae.

If Chatton refers to the Actinocephalidae *s. lat.* of Léger and Duboscq, it is quite possible that he is right. There are, however, two difficulties, which further research may, it is true, dispose of: both sorts of gametes appear to be non-motile, and two sorts of male gametes have not been distinguished.

In view of these difficulties in placing *Agrippina* among the Actinocephalidae, even as a sub-family, the Agrippininae, it seems best to adopt provisionally Strickland's standpoint, and for the present to regard *Agrippina* as the representative of a new family of gregarines.

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Léger, L. and Duboscq, O. (1909). Études sur la sexualité chez les Grégarines. *Arch. Protistenk.* xvii. 19–134.

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## DESCRIPTION OF PLATE XVIII.

Fig. 1. Section through young trophozoite, showing nucleus. Obj. $\frac{1}{12}$ × oc. 6.

Fig. 2. Section through older trophozoite, showing nucleus which contains an achromatic spherule. Obj. $\frac{1}{12}$ × oc. 6.

Fig. 3. Full-grown trophozoite. Intranuclear spherule with dark network. Chromatin less dense. Obj. $\frac{1}{12}$ × oc. 4.

Fig. 4. Full-grown trophozoite. Spherule with very dark periphery, and lighter interior with black granules. Karyosome largely depleted of chromatin. Obj. $\frac{1}{12}$ × oc. 4.

Fig. 5. Full-grown trophozoite. Spherule darker than rest of chromatin. Obj. 7 *a* (Leitz) × oc. 4.

Fig. 5 *a*. Nucleus of 5 as seen in next section. Obj. 7 *a* (Leitz) × oc. 4.

Fig. 6. Full-grown trophozoite (transverse section) showing a common disposition of the chromatin. Obj. $\frac{1}{12}$ × oc. 4.

Fig. 7. Young cyst showing protomerites. Nucleus with little chromatin. Obj. $\frac{1}{12}$ × oc. 6.

Fig. 8. Young cyst (oblique section) showing protomerites.

Figs. 8 *a*, 8 *b*. Nucleus of 8 in succeeding sections. Very little chromatin outside spherules. Obj. $\frac{1}{12}$ × oc. 6.

Fig. 9. Cyst (oblique section). Chromatin granular, spherules very pale, ectoplasm chromatic. Obj. $\frac{1}{12}$ × oc. 6.

Fig. 10. Cyst. Spherules pale, chromatic granules in cytoplasm, ectoplasm chromatic. Obj. $\frac{1}{12}$ × oc. 6.

Fig. 11. Cyst with segmentation spindle and old nucleus. Obj. $\frac{1}{12}$ × oc. 6.

Fig. 12. Nucleus and spindle from other individual of cyst shown in Fig. 11. Relation of old nucleus to spindle. Obj. $\frac{1}{12}$ × oc. 6.

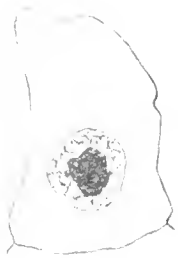
Fig. 12 *a*. Spindle of 12 in next section. Obj. $\frac{1}{12}$ × oc. 6.

Fig. 13. Cyst at later stage, showing mitosis, and "resting" nuclei. Obj. $\frac{1}{12}$ × oc. 6.

Fig. 14. Mitosis seen in cyst at later stage than 13. Obj. $\frac{1}{12}$ × oc. 6.

Fig. 15. Cyst showing staining difference between individuals. Obj. $\frac{1}{12}$ × oc. 6.

All the above figures were drawn from sections stained with iron haematoxylin after fixation in alcohol-acetic. The outlines were drawn with the help of a camera lucida. Except Fig. 15, which represents a section 6 $\mu$ thick, the figures were drawn from sections of thickness 5 $\mu$.



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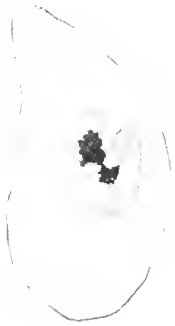

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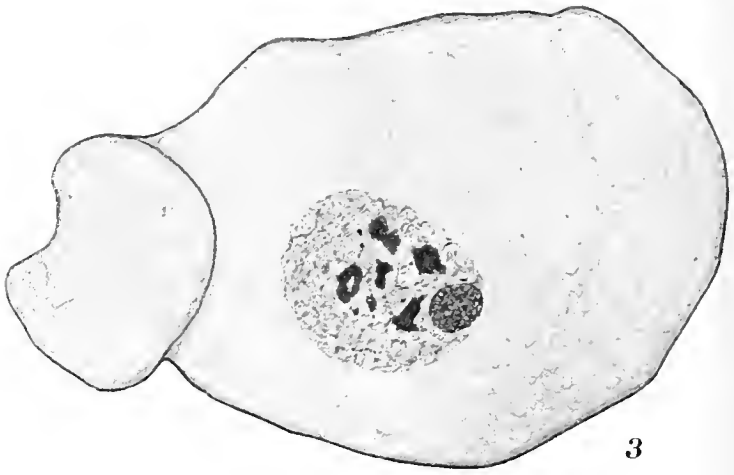

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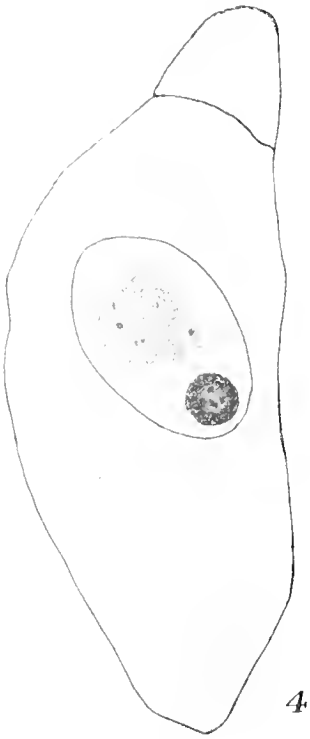

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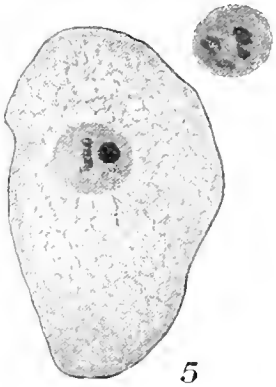

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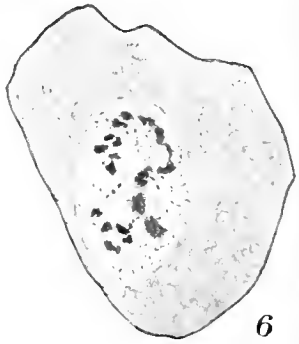

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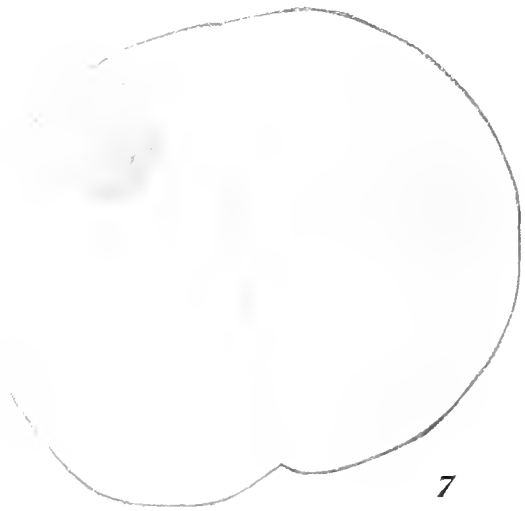

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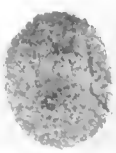

8a

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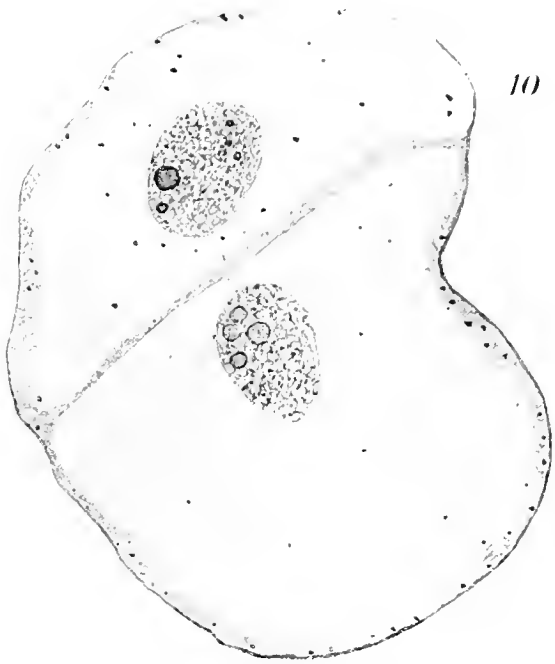

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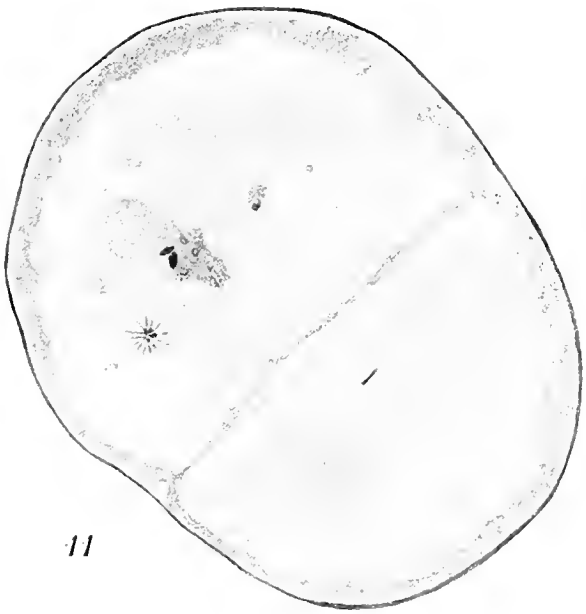

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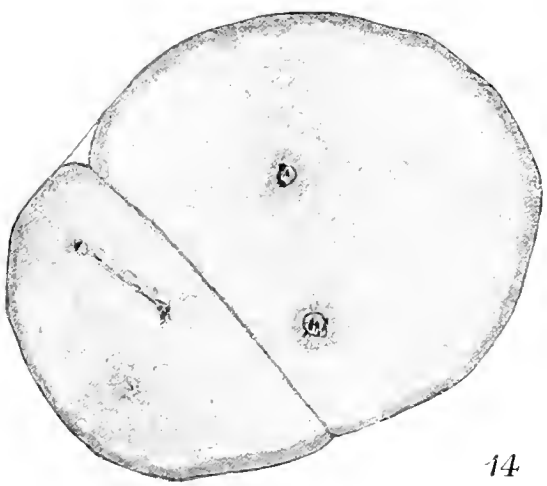

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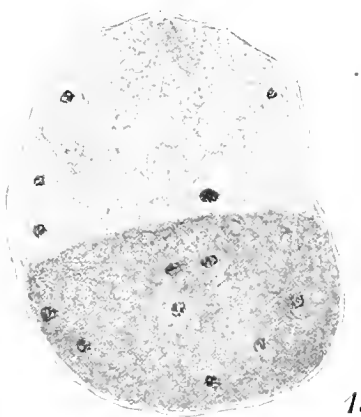


15

# SOME PECULIAR AND PROBABLY SPECIFIC BODIES IN THE ERYTHROCYTES IN RINDERPEST AND ANOTHER ALLIED DISEASE.

By W. LEONARD BRADDON, B.S., F.R.C.S.,

With some observations on the specimens by Col. Sir Wm. Leishman, F.R.S., K.H.P., R.A.M.C., and Professor E. A. Minchin, Ph.D., F.R.S.

(With Plate XIX.)

## I. *Rinderpest.*

The virus of rinderpest is present in the blood and all the body-fluids during the febrile period. In the blood it is so plentiful that the inoculation of a single drop of a 1 in 10,000 dilution is said to be sufficient to infect a calf. According to Nicolle and Adil Bey[1] the causal agent is filterable, and therefore is supposed to be ultramicroscopic. Direct examination of fresh specimens reveals nothing parasitic, nor has anything of the kind been shown by various methods of staining of dried films.

But the writer, who had extensive opportunities for study in connection with an experimental investigation into the production of a protective serum for buffaloes, conducted for the Federated Malay States Government in 1901, found that by the application of a special method which he was in the habit of using in blood-studies, there could be constantly defined in the blood of rinderpest-affected animals certain bodies, not found in controls. The description and illustration of these appearances form the subject of this paper, to which Sir W. Leishman and Prof. Minchin have added the value of their remarks upon the specimens which they have been good enough to examine.

[1] "Études sur la peste bovine." *Ann. d. l'Inst. Pasteur*, Ap. 1899 and Sept. 1901.

*Method.* The blood is diluted with a mixture containing 1 % of citrate of potash, and 0·5 % of ordinary medicinal methylene blue. Different proportions of the fluid must be added, varying from one-fifth to one-third of the volume of blood, according to the condition of the latter, which as taken from the vein may be almost tarry, or excessively hydraemic.

Of the mixture, some specimens are put up under cover-glasses, sealed at the edges with a paraffin of low melting-point; other portions are put in watch-glasses, exposed to the air, but not to light, and are kept in Petri dishes arranged as moist-chambers. When examination reveals the presence of the specific bodies in these preparations, films may be spread in the ordinary way, and fixed as permanent specimens.

*Appearances.*

Forms seen:

(*a*) After an uncertain period, from 6 to 48 hours, there appears upon the blood corpuscle a blue point or dot; later there becomes evident an extension from the dot, in the form of one or more delicate streaks or fine lines going deeper into the corpuscle. Ultimately, by extending and thickening, the lines usually demarcate a long, fine, needle- or sword-shaped body, which may extend into, or across, or even through the corpuscle, projecting from it at either or both of its extremities.

The body may be straight or curved; it may present a single or double contour. Its breadth varies within wide limits. The ends may be pointed or oval. It may lie all in one plane, or be obviously twisted; sometimes it is acutely bent, or even folded double. In perhaps the greater number of instances it lies curved and closely pressed into the periphery of the corpuscle, and in these cases its aspect is such that it might be taken for a mere overstaining of the edge of the erythrocyte. Where the bodies protrude through the wall of the corpuscle they often emerge freely, but in other cases they may carry the membrane with them, causing the corpuscle to become elongated and assume the shape of a fish, or a float. The extreme ends of the bodies when free are sometimes blunt and rounded, but at others extremely tenuous. In length they may exceed two or three times the diameter of the disc. Broader forms occur, often crumpled or folded so as to show two or three ridges or lines of stain. When seen on the flat the edges are extremely faint

They are obviously always excessively thin: and appearances indicate that larger forms may wholly occupy the corpuscle, from the contours of which their own edges are then practically indistinguishable.

The corpuscle may contain one or several of the bodies, clearly distinguishable from each other. Occasionally several lie close together, some perhaps bent, and then present the appearance of a bundle of short lines (like a bunch of twigs) some of which seem to be branched (Pl. XIX, figs. 67, 68).

(Examples of nearly all these variations are given in the plates.)

At first sight therefore the body seems to be pleomorphic, but observation will show that the limits of variation of form are not very extended, and that between the many figures shown, there is no essential difference of structure. The type is that of a slender body, at first almost linear, which may increase in length or in breadth, but always seems to be entirely in one plane. Certain specimens give the impression of a thicker primitive chord or axis, from which the rest of the body grows out as a thin flexible membrane or fringe.

The *protrusion* of the bodies from the corpuscle may be complete, and (generally at a later stage of observation) many elongated and quite filamentous, or broader linear-lanceolate shapes may be seen, just attached by one end to the corpuscle, or floating free in the plasma (Pl. XIX, figs. 22–24, 72–77). Certain of the corpuscles—especially when the blood has been taken near the crisis, in acute infections—show no such forms as the foregoing, but instead, a very minute speckling or stippling of the whole corpuscle with blue, the impression given being that of a mass of granules just on the limits of resolvability, and suggesting the formation of spores or infective granules. More rarely the corpuscles exhibit numbers of larger, deeply staining granules: or yet again similar granules from which may extend comma-like traces (suggesting commencing growth), or small extremely thin and short curved forms (Pl. XIX, figs. 52, 53, 54).

(*b*) The foregoing account is concerned with the appearance of the bodies in stained preparations. But in some specimens—dependent upon conditions the writer has not yet been able to determine—before the presence of the endoglobular bodies has been made manifest by colour, it is indicated in other ways:

(I) "*Squared corpuscles.*" The corpuscles undergo a singular—and to my mind a pathognomonic—alteration of outline. A portion of the contour becomes *straight* while the rest of it remains smooth and round. The disc then appears as if a part of its circumference had been simply

sliced off. Sometimes only a quarter or third of the whole periphery is thus straightened, but at other times the corpuscle is reduced to a plain half-moon shape. At the same time measurements show that nothing has been really lost, the bulk of the corpuscle as a whole is not reduced. This phenomenon of "squared corpuscles" is clearly due to a stretching of their margin by something which lies within. It seems impossible to refuse assent to the belief that this something is the specific body which the stained specimens depict, which by reason of growth, or change in rigidity, effects the distortion. The body when in this state, has in all my specimens proved refractory to stain (Pl. XIX, figs. 81, 82, 84, 87).

(II) *Protrusions*, and *projecting filaments* of variable size and form emerge from the corpuscle. These projections are of highly refractive appearance, uncoloured, or if of any tint, of that of the corpuscle itself. In fresh specimens they are always unstained, and in dried films take on equally whatever stain is absorbed by the haemoglobin. Their projection appears to make no difference to the remainder of the corpuscle, which retains perfectly its colour and contour. As with the stained bodies, these protuberances may become wholly free of the corpuscle, or remain but just attached to it by a single extremity: and many again are to be seen floating freely in the plasma, which do not seem to have been immediately derived from any erythrocyte (Pl. XIX, figs. 72–77 and 88–109).

*Movements.*

1. It is frequently seen that the corpuscles, during the first twenty-four hours or so of observation, and especially when in the condition which I have termed "squared," are in active movement. Rapid oscillations of brief period are combined with rotatory changes of position. The effect is on an exaggerated scale the same as Brownian movement. But with much experience of the behaviour of corpuscles in various fluids I am unable to recall any condition in which the same effect is exhibited. The slow irregular translatory motion of erythrocytes under the influence of contained active piroplasms (as described by Smith and Kilborne in their original monograph) is by no means the same thing. The jostling of the corpuscles in the state now described is very lively, and results in a considerable extent of change of place, or translation among them.

2. Within the corpuscles, the specific forms, even when in a fairly advanced condition of staining, and after several days of observation, exhibit definite movements of their own:

(*a*) They bend and unbend, sometimes pretty rapidly.

(*b*) They twist or rotate sluggishly, about their long axis.

(*c*) When present as short forms they change position freely within the corpuscles.

3. When attached to the exterior of the corpuscle by one end only, the filamentous forms sway freely in the plasma and also vibrate in a limited range to and fro.

4. When wholly free in the plasma the filamentous and slender lanceolate forms move slowly and irregularly through and about the field, exhibiting movements of rotation, and of bending or unbending.

*Relation to the disease.*

The bodies have been invariably found by the writer in all cases of typical acute rinderpest, during the febrile stages, and in the great majority of cases for long periods, up to eight months, after recovery has taken place, a fact of which the significance will be discussed later.

In artificially inoculated animals (proved first to be free from these forms) they appear as early as the third day after infection—slender forms equal to the radius of a corpuscle being by then often present in great abundance. From this date onwards they increase rapidly in both number and in size, until ultimately, even in serum-protected animals, three-quarters of all the discs may be occupied.

The earliest appearances after inoculation are minute dots, and slender comma or short-curve forms. In natural infections, especially of the buffalo (which is unusually sensitive to the disease), every corpuscle may be infested with many of the bodies. Their appearance precedes by several days the onset of pyrexia and thus forms a valuable means of discrimination of contacts.

This was the case for instance in the beast from which the specimen was taken which is illustrated in Pl. XIX, figs. 63–71. All those nine corpuscles were adjacent discs in the same field. The animal appeared on that date perfectly well, but three days after was dead, of the acutest, "algid" type of attack.

In animals less susceptible than buffaloes, such as cross-bred Indian and Siamese cattle, the bodies appear always both less pronounced in size, less active in movement, and less numerous. In the successive reactions following injections of virus in immunised animals, even in buffaloes, there is the same progressive diminution in size and number[1].

[1] Ultimately, whether after natural recovery or at the end of the immunising process, the earlier shapes give place to short thick and stumpy or rounded forms which resemble mere accretions of haemoglobin and stain, only they are seen entirely within a perhaps unaltered corpuscle. (See Plate XIX, figs. 111, 112.)

In one of two domestic *pigs* inoculated with virus, the blood of which showed no such appearances before inoculation, although there was no reaction, numerous slender forms were found in blood on the fourth day (Pl. XIX, figs. 55, 56, 57). In *goats*, which act as passive carriers for virus (which in them is active from about the 4th to 8th days after inoculation), the bodies could not be distinguished in any of the shapes occurring in cattle. The corpuscles of this animal however were observed to take on the unusual aspect figured in fig. 110. At each end of the disc protruded a long filament, resembling those jets of corpuscular substance well known to be produced under conditions of haemolysis, with the difference that there was in these cases no disruption of the corpuscles, and the shapes were maintained unaltered for periods of very many, ten or more, days of continued observation.

Since the bodies persist for long periods after recovery from rinderpest in both buffaloes and cattle it is natural that they should at times be found also in beasts not known to have actually had or to have been exposed to rinderpest. The writer in the course of extensive examinations of supposed healthy control animals, found the bodies fairly frequently in Siamese cattle, but on no single occasion in any buffalo not known to have had or to have been exposed to the disease, nor at any time in buffaloes in districts in which rinderpest had not appeared.

It is known that Siamese cattle are very largely immune to rinderpest. The case-mortality among them during epizootics seldom exceeds one-third, and the herd-mortality[1] probably not one-fifth. They are imported from countries in which rinderpest is practically enzootic. But with the Malay buffalo the case is very different. The case-mortality of undoubted rinderpest among them is often 90 %, sometimes 100 %. And as a rule the herd-mortality is not less severe, since by the time the pest has become recognised, almost every animal in a valley, under the conditions of communal pasturing, has been exposed to infection.

## II. *A new rinderpest-like malady.*

In January 1912, my friend Mr L. V. Symonds, M.R.C.V.S., who is in charge of the Veterinary work in Negri Sembilan, was kind enough to show me some buffaloes, suffering from a disease, forming a limited epizootic, the clinical symptoms, and post-mortem appearances of which, in his opinion, indicated it to be true rinderpest. His own observations

[1] Meaning proportion of deaths to number of animals in a known herd, in a limited area where all are possible contacts.

directed to the discovery of piroplasms, the bacteria of haemorrhagic septicaemia and other known pathogenic elements, had resulted in purely negative findings, which led to the presumption that the affection was rinderpest. The mortality among the observed cases, as well as the attack-rate among contacts, was however unusually low, a suspicious happening in regard to buffaloes not immunised.

Mr Symonds assisted me to procure blood-specimens which I prepared after my usual method, expecting to find the wonted specific bodies which have been described above.

None of these bodies was found. But, instead, every specimen taken showed the corpuscles to be copiously infested with a new and totally different form of inclusion, illustrated in Pl. XIX, fig. *f*.

This new element exhibited short thick ovoid, or round, angular or sometimes almost square shapes, of which one or many might be found in nearly every corpuscle. Their colour as stained was deep green, or blue to black. Their size varied from $\frac{1}{2}\,\mu$ to $3\,\mu$. In structure they seem to consist of a vesicle which is filled and possibly (through absorption from an anisotonic medium) distended with stain. One peculiarity is a tendency, like that of the specific bodies of true rinderpest, to protrude from the corpuscle. Many of the bodies are seen thus almost entirely extruded, but none apparently quite free.

Unlike the other bodies they retain their first blue stain well when washed in alcohol: but in these, as in the others, the writer has so far been unable to show any evidence of chromatin, or indeed any special or selective staining affinity.

*Remarks as to technique.*

The writer's specimens were all stained with a mixture of $\frac{1}{2}\,\%$ methylene blue and $1\,\%$ potassium citrate; but it seems reasonable to suppose that the soda salt would serve equally well. With normal saline solution however (and the blue) repeated attempts failed to yield any staining effects.

When the fluid specimens are sufficiently stained they may be spread on a slide and fixed by formalin or osmic vapour, or saturated corrosive sublimate solution—the latter giving the best results.

In this state the margins of the bodies are seen very clearly outlined. If now any of the common plasmatic stains (Fuchsin, Eosin, Methyl violet, Thionin, Safranin) be applied, the effect is speedily to dissolve out the original blue, leaving the included body wholly undemarcated, and therefore invisible. Safranin, and Thionin in watery solution, applied

for a few moments only, accentuate the Methylene blue contours; but a $\frac{1}{2}$ % solution must not be allowed to act longer than two or three seconds. The various methylene blue-and-eosin compounds (Giemsa, Romanowsky, Leishman), owing to the alcohol, all dissolve out the blue, and do not replace it. The bodies are thus washed out. Carmine and Iron Haematoxylin are equally useless. For a long while the writer had been unsuccessful in the search for a means of staining more than the margins of the bodies; but the following procedure reveals the surfaces lying between these margins. The specimen having had any excess of the mercury salt and of blue washed off, is soaked for five minutes in the original blue solution, and overstained. Examined at this stage the corpuscles are of a dense blue, and the bodies within them invisible. Wash, and stain in a mixture of Orange G. 2 %, Anilin blue 1 % and Oxalic acid 2 % in water, for ten seconds only. The corpuscles and contents are rendered deep chocolate, the bodies still invisible. Wash, first with water, then with Absolute Alcohol, then with Acid Alcohol, then again with water. The alcohol washings must be brief, as they rapidly remove the stain. The result, in successful specimens, is to leave the corpuscle green, with the specific body very clearly shown within, of a deep brown colour (Pl. XIX, figs. *b–e* shows the form, the brown colour not being represented).

As has already been mentioned, by no one of very many methods tried by the writer has it been found possible to demonstrate the bodies in ordinary dried films: the application of the stain to them while yet the corpuscle is unfixed, as in the citrated solutions, seems to be *essential.*

## Conclusions.

1. The occurrence of a body of special, and within certain limits, uniform morphology has been demonstrated in the red corpuscles of animals affected with rinderpest.

2. The movements of the body, and the evidence of its growth *pari passu* with the development of the disease, and above all its reproduction in animals in which it was not previously present, on the inoculation of material containing it, are evidence of its being a living and independent organism.

3. Its detected presence (so far) only in animals which at the time have, or which probably have had rinderpest recently, and its entire absence from animals highly susceptible to the disease, but known not to have had, or to have been exposed to infection, affords a presumption

that the body is specifically related to the disorder, or in other words represents a stage in the life-history of the specific infective agent; or, it may be, a culture form.

4. Its presence, in atrophying form, in the blood of immune or recovered animals suggests that it assumes, after the first production of toxic symptoms, a passive (resting, or possibly gamete ?) stage—which may or may not play a part in the revival of rinderpest after passing through an as yet unknown cycle of development.

5. The specific body resembles no parasite of which the life-history is so far known.

Since the specific bodies can be detected in the blood of infected animals before clinical signs of rinderpest are observable, it is possible to arrive at an early diagnosis. This is of great importance in dealing with contacts and the early cases which usher in an outbreak.

6. The second body described affords evidence of the existence of a second specific complaint which may be and probably has been in the past confused with true rinderpest. It would be important to determine if animals affected by the second complaint when they have recovered, are still susceptible to true rinderpest.

7. The second body also is a new form.

The writer offers acknowledgments to Prof. Sir W. Leishman, and Prof. Minchin, who have kindly examined his specimens, and have encouraged him to make this communication. The drawings to which these gentlemen refer, in the notes appended, are lettered *a–f* on Pl. XIX and were made from fixed specimens by Miss Rhodes, at the Lister Institute. The remaining figures are reproduced by Miss Rhodes from my drawings of fresh specimens.

## Note by Colonel Sir William Leishman.

I am much indebted to Dr Braddon for his kindness in letting me see some of the blood films containing the curious bodies which form the subject of the above communication, and I accede with pleasure to his request to add a word or two to his paper.

Naturally, I can do little more than record my impression of the inclusions in the red corpuscles which he has found in rinderpest blood and in the blood of animals suffering from a disease resembling rinderpest. These inclusions were numerous and obvious in the stained films which I studied, and those which I saw, as well as the coloured sketches

accompanying the article, testify to the accuracy of Dr Braddon's description. I have not, personally, seen anything resembling them in any human or animal blood which I have examined—though it should be added that I have no experience of the staining technique employed by Dr Braddon. Although I was at first inclined to regard them as artefacts, due to the staining methods employed, on closer inspection of a considerable number of the inclusions I formed a strong impression that they were not explicable on such grounds.

The inclusions in the red corpuscles of the rinderpest animals are certainly sufficiently remarkable to demand that careful search should be made for them, following Dr Braddon's methods, in countries where this disease is present, and also in the same animals in non-rinderpest countries, in order that there may be sufficient control evidence that the inclusions are actually limited to animals which are suffering or have recently suffered from this disease.

Should this extended investigation confirm Dr Braddon's remarkable results I should certainly be inclined to suspect the inclusions of being both parasitic and etiologically connected with Rinderpest, in spite of the fact that they bear no trace of resemblance to any bacterial or protozoal parasite with which I am familiar.

### Note by Prof. E. A. Minchin.

Dr Braddon has shown me his preparations of rinderpest blood and I have been able to see clearly the appearances represented in the figures, drawn by Miss Rhodes. I have the impression of bodies, for the most part like elongated rods, but somewhat irregular and variable in form, lying in the red blood-corpuscles and sometimes projecting from their surface.

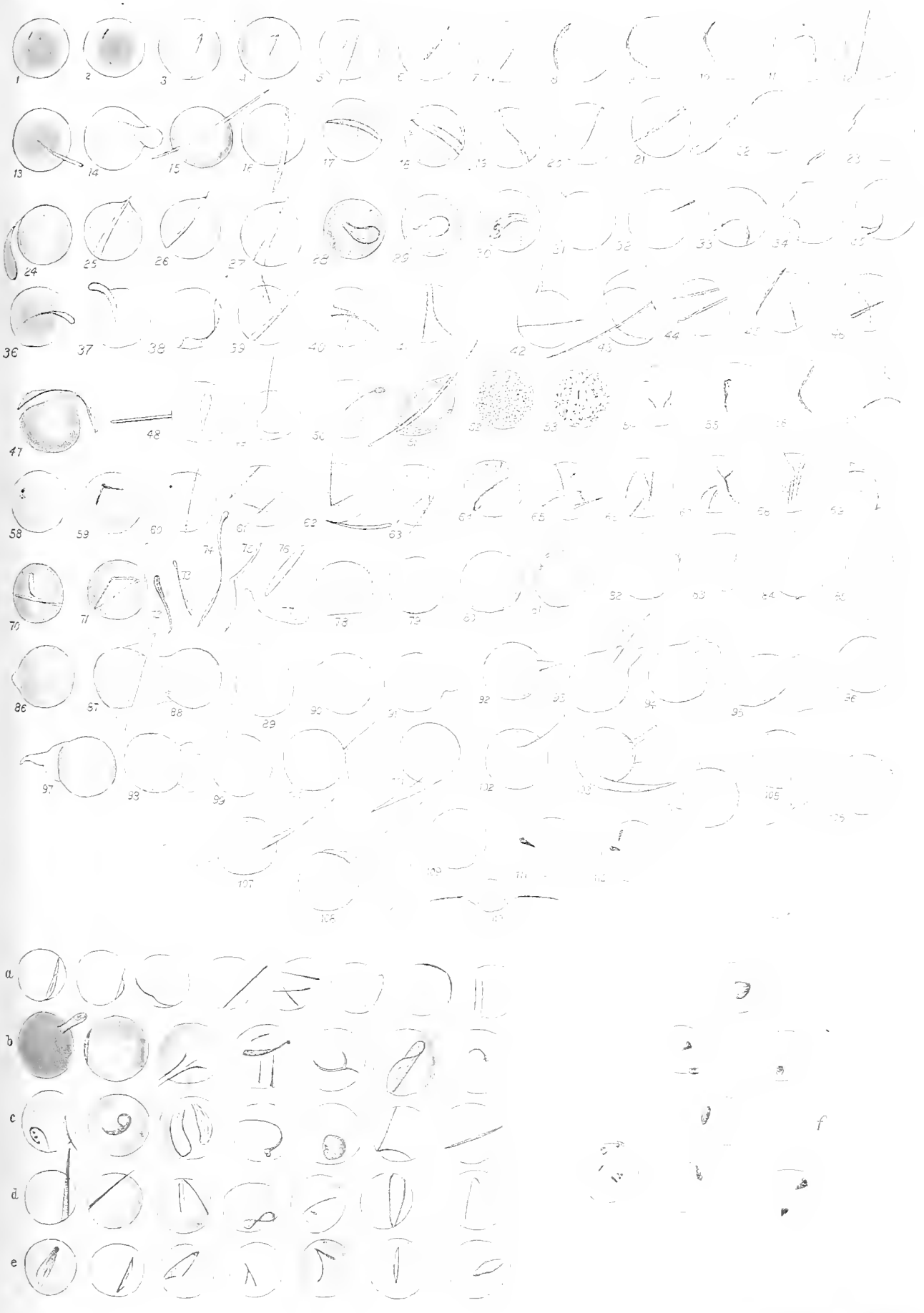
I am not prepared, however, to express any opinion as to the nature of these inclusions in the red corpuscles. They do not resemble anything with which I am familiar amongst the Protozoa.

## DESCRIPTION OF PLATE XIX.

Figs. 1–112. Intra- and extra-corpuscular bodies seen in the blood of animals (buffalo, cattle, pig, goat) affected with *rinderpest*. Blood stained with methylene blue citrate of potash solution and examined whilst fluid. The figures reproduced by Miss Rhodes from drawings of fresh specimens made by the author.

Figs. 1–4. Appearances on the 3rd–5th day after infection (buffalo).

Figs. 5–13. Examples of forms commonly seen in buffaloes.



Figs. 13–16. Some larger forms, partly extra-corpuscular. Note in 15 complete "transfixion" of erythrocyte.

Figs. 18–21. A single body drawn at intervals of 10 secs., showing rotation on long axis.

Figs. 22–24. Almost completely detached body drawn at intervals of 10 secs.

Figs. 25–27, and 28–30. Other single intra-cellular specimens drawn at similar intervals.

Figs. 31–34. Slender forms (stunted) from blood of partially immune Siamese cattle.

Figs. 35–51. Various examples from buffaloes.

Fig. 52. From buffalo during infective stage. Appearance suggesting full-grown body completely filling corpuscle, converted into granules at limits of resolvability (the dots in the drawing are too large)—infective granules?

Fig. 53. Cell full of larger (growing?) comma forms from buffalo during infective stage, 5th day.

Fig. 54. Multiple young forms from buffalo, early stage of infection.

Figs. 55–57. From pig artificially inoculated with virus from buffalo, five days after infection.

Figs. 58–60. Buffalo, fourth day after inoculation.

Figs. 61–70. Multiple forms, from a single field, in blood of a buffalo-contact, which at date of examination appeared healthy (no temperature) but three days after was dead of an "algid" (hyperacute) attack of rinderpest.

Fig. 71. Scalene triangle form, very common. Note only margins of inclusion are indicated, membrane between them unstained.

Figs. 72–77. Free forms, commonly seen in plasma in buffaloes after 4th or 5th day of infection; stained and unstained.

Figs. 78–109. Red corpuscles with distortions indicating presence of specific inclusions, but in which no staining having taken place they are not defined.

Figs. 78–84. Typical "squared" corpuscles. These are often seen within 12 hours and, before staining, are pathognomonic as indicating presence of specific inclusions.

Figs. 79, 80, 83. Normal red corpuscles drawn beside the squared corpuscles for comparison.

Figs. 85–109. Various shapes assumed by extruding specific bodies in buffalo blood. These are refractory to stain in the fluid condition, but take on all plasmatic stains equally with the haemoglobin in fixed specimens.

Fig. 110. Form assumed by red corpuscles of goats infected with rinderpest.

Figs. 111, 112. Late atrophied forms of intra-corpuscular bodies in buffaloes eight months to a year after recovery from rinderpest.

Figs. *a*—*f* were drawn from actual specimens by Miss Rhodes at the Lister Institute.

Figs. *a*—*e*. Intra-corpuscular *bodies occurring in rinderpest*. Blood stained, while fluid, with methylene blue citrate of potash solution, and afterwards fixed and restained.

Fig. *a*. Slightly counterstained with Eosin (colour omitted in plate). Only the edges of the inclusions appear to be stained.

Figs. *b*—*e*. After treatment with Orange G. and Anilin blue. A definite expanse of membrane between the edges is shown. (In the original figure the inclusions appear brownish.)

Fig. *f*. Blood from *a case resembling rinderpest*. Group of corpuscles in a microscopic field. Blood fixed in perchloride of mercury after staining, while fluid, with methylene blue citrate of potash solution.

# OBSERVATIONS ON MAMMALIAN ERYTHROCYTES.

By J. CHARLES JOHNSON, M.A., M.Sc., M.B.

(*From the Quick Laboratory, University of Cambridge.*)

This brief note is the result of a series of examinations of blood-films of various mammals and was suggested by Dr Braddon's interesting paper, the manuscript of which I had an opportunity of reading while working in the Quick Laboratory, Cambridge[1].

The object of the research was to observe the red blood corpuscles of as many animals as possible, both healthy and suffering from blood-disease, with a view to finding any appearances similar to those described by Braddon as specific to rinderpest.

*Material.* Owing to the kindness of Prof. Nuttall the blood of several of the animals on which he is experimenting at the University farm was placed at my disposal. The following is a list of the sources from which blood was taken for observation.

| Normal | Abnormal |
|---|---|
| Man | Calf with East Coast fever (*Theileria parva*) |
| Two dogs | Three dogs in various stages of acute piroplasmosis (*Piroplasma canis*) |
| Two rabbits | |
| White rat | Two dogs in different stages of chronic piroplasmosis (*P. canis*) |
| Calf | Horse with African biliary fever (*Nuttallia equi*); "salted" animal |
| Ram | |

*Method of collecting.* The ear was usually selected as the site of the blood-letting. After preliminary shaving and swabbing with spirit a vein was opened with a sterilised needle or scissors.

The blood was at first collected in watch-glasses containing various quantities of the staining fluid used by Braddon in his research (methylene blue ·5 % and potassium citrate, 1 %). The watch-glasses were

[1] Dr Braddon's paper appears in this number of *Parasitology*.—Ed.

placed in Petri dishes arranged as moist chambers and kept in the dark. Having had to make so many preparations I found this method rather inconvenient, besides not gauging the dilution of the blood definitely, and abandoned it for the collection of the blood in tubes of small bore. A certain amount of the methylene-blue-citrate mixture was placed in the tube and the blood was allowed to run in until it reached a mark previously made on the tube. Several specimens were thus taken from each animal containing blood diluted from one-sixth to several times its volume with the staining solution. Great care was taken that no clot should form on the skin of the animal, and as the blood entered the tube the latter was gently shaken to distribute the corpuscles. The tubes were protected from light. In every case at least one specimen of the blood was run into tubes containing Hayem's solution for microscopical comparison with blood mixed with methylene blue.

In the normal animals I found the quantity of stain recommended by Braddon (from one-fifth to one-third volume of blood) to be inadequate in preventing coagulation. Thus, one-fourth dilution of rabbit's blood produced a distinct but delayed clot while the same dilution of the more hydraemic blood of a dog with acute piroplasmosis produced none.

*Examination.* Four methods of examination were used in observing the condition of the erythrocytes:—

1. A drop of the mixture from the tubes was transferred to a slide, covered, and evaporation prevented by means of vaseline around the edge of the cover-slip.

2. Cover-glass films were made from the tube specimens, fixed wet over formalin, or with a saturated aqueous solution of mercuric chloride, and finally mounted in Canada balsam.

3. Films of the fresh blood were made at the time of collection, and treated with either Giemsa's or Leishman's stain.

4. For correlation with the above, unstained films were observed of the blood mixed with Hayem's solution.

Specimens that had clotted were, of course, useless for microscopical observation, and in addition to these a great many films had to be rejected owing to the osmotic changes in the red corpuscles. Many varieties of dropsical, crenate, buckled and gastrula-shaped erythrocytes were found in preparations in which isotonicity had not been preserved.

The logical objection to the method of blood-collection as carried out above lies in the uncertainty of knowing whether the right amount of

stain has been mixed with the blood to retain the histologieal eharaeters of the latter.

In speeimens in whieh the latter eondition was fulfilled I found the solution used by Braddon to be an admirable preservative for blood-films; several preparations "ringed" with vaseline kept in perfect eondition for over a month. This solution also brought out the parasites and degeneration in the diseased states but, naturally, did not differentiate the contents of the erythroeytes so well as Giemsa's or Leishman's stains.

After prolonged examination of many films I was led to eonelude that

1. The appearances of erythrocytes in the morbid eonditions examined were easily differentiable from those described by Dr Braddon.

2. That normal blood corpuscles from the above sourees presented nothing like his deseription of the alleged parasite of rinderpest, whieh tends to support his view that what he describes is speeific to that disease.

# TRI-RADIATE *TAENIA CRASSICOLLIS* RUD.

By S. O. YOSHIDA.

(*Zoological Institute, Science College, Imperial University, Tokyo, Japan.*)

(With Plate XX.)

Among other cestodes collected by me some years ago were specimens of the prismatic malformation of a tape-worm which I have recently examined. This abnormality in the taenioid group of cestodes has been frequently reported in Europe, especially in *Taenia saginata*, since Andry's case was first described in 1700. This malformation is known as *Taenia* 'tridre,' 'triquestre,' or 'prismatique' in French; as 'dreikantige' in German, and as 'tri-radiate' or 'prismatic' in English. But as far as my knowledge goes, we do not find a full description of any such abnormality in *Taenia crassicollis*, though a brief description of it was given by Bremser in 1819 and 1824. It is now recorded for the first time from Japan.

The material upon which the following description depends was sent by the teacher of a certain secondary school in the Province Shinano, the middle part of Japan, who collected it from the intestine of a house-cat. The total length of the specimen is 220 mm., and its segments are about 240 or more in number. The length of the segments gradually increases posteriorly, reaching a maximum of 7 mm. at the last segment, while the first segment only measures 0·1 mm. in length. The width (of each wing of the prismatic worm) of the first segments is also small, about 0·6 mm., but rapidly increases posteriorly and attains its maximum width of 2·5 mm. at a distance of one-fourth of the total body length from the head. This width continues nearly throughout the middle third of the body, and then it gradually diminishes toward the posterior extremity where the width is 1·5 mm.

The length and the width of segments in various parts of the worm are given in the following table:

| | Last segment | 10th segm. from last | 20th „ | 30th „ | 40th „ | 50th „ | 70th „ | 80th „ | 90th „ | 100th „ | First segment |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Length | 7·0 mm. | 4·0 | 3·0 | 2·5 | 2·0 | 2·0 | 1·5 | 1·0 | 0·75 | 0·5 | 0·1 |
| Width | 1·5 mm. | 2·3 | 2·5 | 2·5 | 2·5 | 2·0 | 2·0 | 2·0 | 2·0 | 1·5 | 0·6 |

The above table shows that the segments successively increase in length from the anterior to the posterior end, but the width of 2·0–2·5 mm. persists throughout the greater part of the body except for short portions at both ends.

Each wing of the prismatic tape-worm in question is similar in all respects, that is, in size, form, length, width of segment, and in radiation of the angle of the wings from the axis of prism, so that a cross section would have the appearance of a regular tri-radiate star.

The scolex (Pl. XX, fig. 1) measures 1·5 mm. in width and, instead of four, is provided with six suckers grouped into three pairs, each of which is situated on the anterior end of each wing. All of the suckers are equally prominent and turn forward and outward.

The hooks are 42 in number, arranged in two crowns, the hooks of one circlet alternating with those of the other, as in the ordinary *T. crassicollis*.

The internal structure does not need a detailed description as it agrees in its main characters with that of normal individuals of this species. I shall therefore restrict myself to a brief description of the excretory system and such of the genital organs as are more or less peculiar and different from those of normal specimens.

In each wing of the tri-radiate worm, the two main longitudinal canals of the excretory system lie near the lateral side of the medullary portion, just inside the nerves. The two canals, instead of being situated dorsally and ventrally and superposed, run side by side, as in the ordinary *T. crassicollis*, and as is often the case in other forms. The outer canal is the larger, but the small canal possesses a more muscular wall. It is obvious that the former is the ventral, and the latter the dorsal canal, both from analogy with other forms and from the connexion of the transverse canal with the larger one; I shall refer later to a peculiar mode of this connexion. The ventral canals in the three wings are connected with each other by the transverse canals at the posterior end of each segment. In cross sections of this region of a segment, we find the three transverse canals radiating into each wing from the axis of the prismatic body (Pl. XX, fig. 7, *tv.*). The connexion between the ventral

and the transverse canals is peculiar, the outer end of the latter dividing into two halves and each of the halves passing respectively dorsally and ventrally on each side of the dorsal canal, and opening separately into the ventral vessel. This unusual mode of connexion was recently observed and described by Beddard (1912) in *Urocystidium gemmiparum.*

The male reproductive organs consist of the testes, the vas deferens, the cirrus pouch, and the cirrus. The testes are oval or sometimes globular in shape arranged in two or three rows and fairly equally distributed in all three wings. In normal specimens of *T. crassicollis* the testes are confined to the dorsal side of the segment, but this is not the case in the tri-radiate form (fig. 8).

The vas deferens (fig. 9) is much coiled, and, though small in diameter, it forms a voluminous mass when fully distended with spermatozoa. Entering the cirrus pouch at its base the male duct coils once or twice upon itself, then runs directly outward to open into the genital cloaca. The distal straight portion of the male duct in the cirrus pouch is termed the cirrus; its wall is more muscular than in any other part of the male duct. The cirrus pouch is a long, slender, cylindrical sac surrounded by muscular walls and its base reaches nearly to the excretory canals which are situated dorsally and ventrally of it.

The female organs consist of ovary, yolk-gland, shell-gland, vagina, and uterus. The ovary is irregularly lobed, situated on the basal portion of each wing near to the posterior end of each segment (fig. 6, *ov.*). The shell-gland is spherical in shape and occupies the centre of the axis at about the same level as the ovary (fig. 6, *sg.*). The yolk-gland lies a little behind the shell-gland.

In the anterior younger segments, the uterus consists of a mere longitudinal cavity running anteriorly from the shell-gland, in the axis of the worm; in the mature segments, not only does the main axial trunk of the uterus become extended, but it sends out laterally long and numerous branches into all three wings. Such branched lateral portions of the uterus may be easily distinguished from the radiating, transverse commissures of the excretory system by the presence of the uterine eggs in their cavity (figs. 7 and 8).

From the common genital cloaca, the vagina runs straight inwards parallel with the cirrus pouch and the vas deferens to the inner end of a wing, where it bends posteriorly to run to the region of the segment where the ovary lies. The vagina is small in diameter, but with thicker muscular walls than the vas deferens, and they are easily distinguished from one another even in the same part of the body.

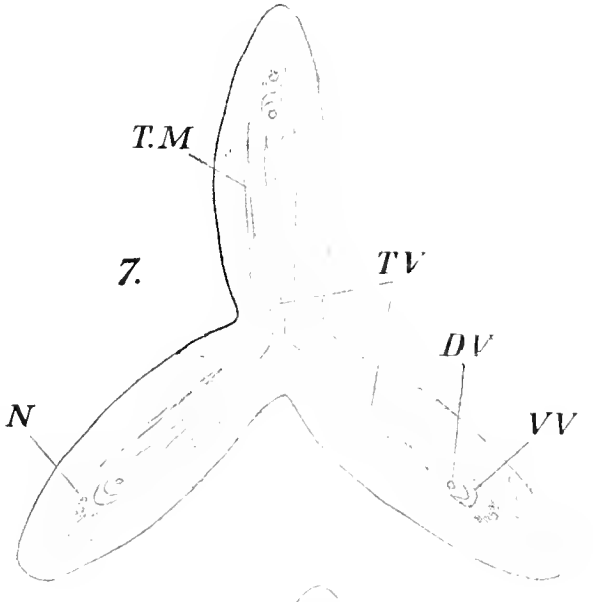
Both vagina and vas deferens pass ventral of the longitudinal excretory canals and nerves on their way to the exterior. The genital pore is usually single in each segment, situated on any one wing of the worm, but there are, sometimes, two genital pores lying respectively on any two wings of a segment.

There is no need to mention in detail the other internal structures such as the musculature, nervous system, abundant presence of the calcareous corpuscles, etc., because they agree in all respects with those of normal *T. crassicollis*.

## EXPLANATION OF PLATE XX.

Lettering:—*C*, cirrus; *cp*, cirrus pouch; *D*, yolk-gland; *DV*, dorsal canal; *H*, testes; *N*, nerves; *OV*, ovary; *Sg*, shell-gland; *TM*, transverse muscles; *TV*, transverse canal; *Ut*, uterus; *Vd*, vas deferens; *Vg*, vagina; *VV*, ventral canal.

Fig. 1. Scolex and anterior segments. (×15.)
Fig. 2. 100th to 120th segments from the last. (×2.)
Fig. 3. 50th to 57th segments from the last. (×2.)
Fig. 4. 20th to 26th segments from the last. (×2.)
Fig. 5. Last 7 segments. (×2.)
Fig. 6. Central portion of cross section at the level of female glands.
Fig. 7. Cross section of posterior end of segment showing the connexion of transverse and ventral canals of excretory system.
Fig. 8. Cross section of mature segment showing testes and branched uterus.
Fig. 9. Cross section of segment at the level of cirrus pouch.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7.
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N
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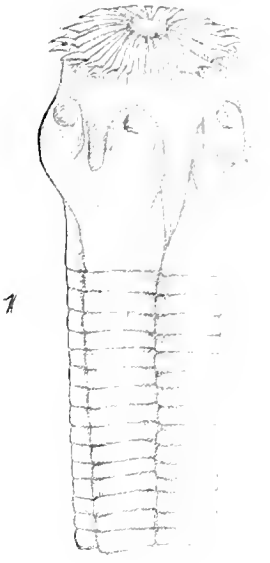
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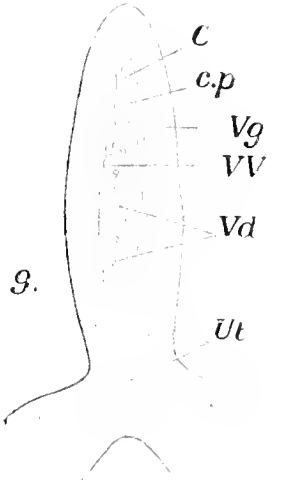
C
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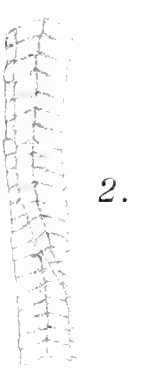
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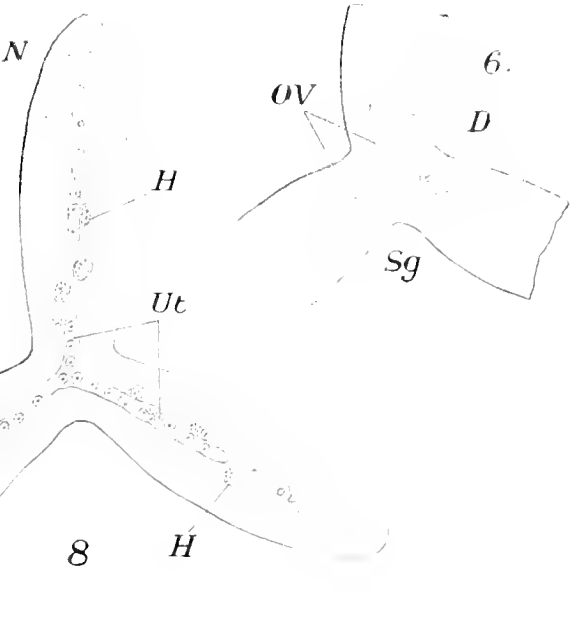
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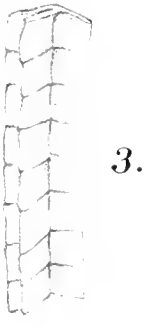
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BRITISH MUSEUM
NATURAL HISTORY

# ON "TICK PARALYSIS" IN SHEEP AND MAN FOLLOWING BITES OF *DERMACENTOR VENUSTUS*.

## WITH NOTES ON THE BIOLOGY OF THE TICK.

By SEYMOUR HADWEN, D.V.Sci.,
*Pathologist to the Dominion Department of Agriculture, Experimental Farm, Agassiz, B.C., Canada.*

(With Plates XXI and XXII.)

### *History of the disease.*

For the past three years, 1910 to 1913, complaints have been received from a sheep farmer living near Keremeos, British Columbia, about a form of paralysis affecting his sheep and occurring annually from late February to early April.

The history of the disease is as follows:

In the autumn of 1910 he imported 900 sheep from Montana; all went well until the middle of March, 1911, when his sheep began to show symptoms of paralysis. During March and up to April 7 he lost 46 old sheep.

In 1912, from March 1 to 15, 34 yearling sheep were ill, but no old sheep; 10 head died. Later in the year (May, June and July), many more were affected—about 300 in all—and 80 died, but it is impossible to tell exactly what ailed them. Veterinary-Inspector Germyn, however, saw some of the diseased sheep and reported that they were suffering from a form of paralysis.

In 1913, from March 25 to April 5, five yearlings were paralysed, but none died.

Acting under instructions from my Department, I visited this district in April. I was unfortunate in not seeing any recent cases, but

probable. Nuttall (1908) mentions the fact that the bites of the nymphs of *Ornithodorus moubata* are more to be feared for their after-effects than the bites of the adults, quite apart from the transmission of the disease.

In referring to the paragraph on the history of the disease, it will be seen that the old sheep became paralysed the first year and that only yearlings were attacked in subsequent years, showing that an immunity is produced after one attack. This does not interfere with the toxin hypothesis, for examples of immunity following the repeated bites of other arthropods (mosquitoes, etc.) are common. Rocky Mountain fever is the only other disease known to be transmitted by *D. venustus*, and at first it seemed possible that there might be some connection between the two diseases, but so far there seems to be no evidence of the occurrence of Rocky Mountain fever in British Columbia.

The symptoms of "tick paralysis" in lambs develop gradually. The first noticeable sign is restlessness, the lambs at times stagger about and bump against obstacles and occasionally fall when trying to stop; later on they fall down and cannot rise; at this stage they struggle a great deal. As paralysis advances the lambs cease struggling but still have a wild-eyed look; they drink milk with less avidity. As soon as recovery begins they again continually struggle in their attempts to rise, and this continues until they are able to stand, after which recovery is very rapid.

No efforts have as yet been made to treat the affected animals, but this will be undertaken in the course of further investigations. It would appear advisable, however, on general principles, to remove the ticks from affected animals and to resort to dipping, if possible.

### *The local effects of the bites of* D. venustus.

The ticks are easily found on newly shorn sheep, because soon after a tick becomes attached it voids excrement which collects and dries in the hair, thus forming a dark reddish patch. Male ticks frequently attach and detach themselves, and whenever they do so bleeding is profuse; the blood seems to have lost its clotting properties. In Pl. XXI, fig. 1, the effects of the bites under a lamb's skin are visible. The haemorrhages have some resemblance to those seen in cases of haemophilia. In man, shortly after being bitten, a scarlet, irritable patch appears. I was once bitten on the neck by a male. The bite was not

felt at first, but the movements made by the tick in detaching itself drew my attention to it; bleeding was profuse, and did not stop for some minutes.

In sheep, after a few days, a bruised appearance develops about the seat of the bite and persists for some time.

*Position of ticks on the host.*

Out of 108 ticks collected on about 25 sheep on the range, all but four of those which had attached themselves were situated along the back-bone (one on the brisket, three round the base of the ears). The sheep were not all examined very carefully, but a number were turned over and the belly and legs searched. Many of the ticks were not attached; they were found loose on the fleece and had evidently been picked up recently.

In my experiments the ticks became attached as follows:

On lamb I, ten ticks attached themselves within half-an-inch of the back-bone on either side, and one at the base of the right ear (Pl. XXI, fig. 2).

On lamb III, three females attached themselves along the back-bone, one male at the base of the right ear, and one female on the brisket.

On lamb IV, two females and one male attached themselves along the back-bone (Pl. XXI, figs. 3 and 4).

When first applied the ticks were scattered along the back and their movements were not interfered with. Only in one case, that of lamb III, were ticks (one male and one female) placed in position and watched until they attached themselves. This was done three days after the first were put on, as only two had become attached in the lumbar region. It is possible that the second lot of ticks had nothing to do with producing paralysis, for the female was barely gorged when the symptoms first appeared.

The reasons for *D. venustus* choosing the back and neck as its seat of attachment are probably as follows: (*a*) there is frequently a parting in the wool along the back, especially in Merinos; (*b*) the ticks are likely to be brushed off on to the backs of sheep when the animals pass under bushes; (*c*) all the denuded portions of a sheep's body are greasy; (*d*) the wool is very thick and close on all other parts of the body, except on the brisket, where ticks are occasionally found attached.

*Regions where ticks attach themselves on other animals and on man.*

In raising experiments on *rabbits* the larvae and nymphs seem to attach themselves almost anywhere ; perhaps the most favourite places are around the muzzle, eyes and the neck. As no larvae or nymphs were found in these experiments they are not considered, and my remarks refer to adults only.

In *horses* and *cattle* the neck, withers, and the perineal region seem to be favourite localities for attachment. In man the nape of the neck is undoubtedly the favourite place—all the shepherds, miners and trappers I have interviewed say the same thing. Medical men also refer to this peculiarity.

*Proportion between the sexes found on sheep.*

Out of 87 ticks collected off sheep on the range, 53 were females and 34 males.

*Habits of male ticks.*

When males are placed on sheep, their first impulse is apparently to feed, and in these experiments they did not seem to pay any attention to the females. They reattached themselves several times, and after three or four days sought out the females; then they fastened themselves quite close to them and copulated. The males are somewhat distended after feeding—not at all like *Ixodes*—and judged from the limited number of observations I have made, copulate with semi-gorged females. This may be different in nature. Males and females will attack a host at different times, and in this event the females may be impregnated before gorging.

As males attach and reattach themselves to a host regardless of females being about, it makes them decidedly more dangerous to man than the males of many other species. Whether they can produce paralysis in the absence of females remains to be proved, but certainly with regard to spotted fever in Montana this habit on the part of *D. venustus* doubtless renders them more dangerous.

## *Protocols of experiments.*

*Tick-transmission experiment* (14–25 IV. 1913).

*Lamb No. I.*

| Day | |
|---|---|
| 1 | Put eleven ♀s and two ♂s *D. venustus* on lamb's back. |
| 2 | One ♀ fell off without becoming attached. |
| 3 | Eleven ticks attached themselves, all but one (on back of ear) attached within half-an-inch of the median line on either side. |
| 5 | Lamb not drinking his milk as usual in the evening. |
| 6 | Lamb weak. |
| 7 | Loss of coordination becoming marked as day progressed; at 4 p.m. could not stand. Two gorged ♀s dropped off. Temperature 103·1° F. |
| 8 | Total paralysis, except for head and neck; lamb feeds well notwithstanding; continually grinds his teeth. Temperature 103·1° F. |
| 9 | Paralysis continues. |
| 10 | Temperature 103·2° F. Incontinence of urine since paralysis started; cannot stop flow if tapped or startled. Drinks milk well. |
| 11 | Temperature 103·3° F. Observed ticks copulating on lamb. Paralysis continues. |
| 12 | Lamb raised his head easily in the morning; was much better in afternoon; could stand for a moment when raised; also had more or less control over his urine when startled. |

*Autopsy.* Lamb killed by bleeding. Organs apparently normal. Coverings of the brain congested; a fibrinous exudate present in the ventricles.

*Attempts to transmit the disease by inoculation* (1913).

*Lamb No. II.*

| Date | |
|---|---|
| 25 IV. | Inoculated in three places (lumbar, cervical and occipital regions), with material from brain, spinal cord and cerebellum of Lamb No. I. |
| 28 IV. | Temperature rose gradually to 105·5–106° F. Lamb had soreness between shoulder blades; temperature probably due to the formation of pus. |
| 3 V. | Abscess broke; lamb better. |
| 3–13 V. | Lamb perfectly well. |
| 13 V. | Lamb was reinoculated with mashed-up ticks (three gorged ♀s and one ♂) which were removed from Lamb No. III. |
| 13 V.–1 VI. | Lamb perfectly well; no effects from inoculation. |

*Tick-transmission experiment* (5–15 V. 1913).

*Lamb No. III.*

| Day | |
|---|---|
| 1 | Put seven ♀s and three ♂s *D. venustus* on lamb's back. |
| 2 | Temperature 103·3° F. |
| 3 | Temperature: M. 103° F., E. 103·4° F. |

Day

4 Temperature: M. 102·7° F., E. 103° F. Put on one more ♀ and one ♂ collected from *bear* by J. W. Frank, Creston, British Columbia. These ticks were placed on the neck. Two ♀s attached themselves to the lumbar region, one ♂ on the side of the head, and one ♀ on the sternum.

5 Temperature: M. 102·8° F.

6 Temperature: M. 102·5° F., E. 102·8° F.

7 Temperature: E. 103·6° F. Lamb well in morning; at 9 p.m. showed some loss of coordination; bumped into obstacles when moving about.

8 Temperature: M. 102·8° F., E. 103·4° F. Paralysis advancing, struggles a good deal; if braced could just stand in morniug. The two ♀s attached in the lumbar region; all parts posterior seemed to be most affected. The lamb threw his hind parts in the air, though later iu the day paralysis was almost complete except for the head and neck. Lamb wild-eyed and restless, appetite diminished; one ♀ fell off. Pl. XXII, fig. 5.

9 Lamb could just stand in morning; much improved in afternoon; stood more easily and could stop flow of urine. One ♀ dropped off; two ♀s and one ♂ removed.

10 Lamb much better. Appetite improved; still unsafe on hind legs.

11 Lamb well, except for slight crouch when walking.

12–27 Lamb recovered completely. Killed on day 27; all organs appeared normal.

*Tick-transmission experiment* (5–13 v. 1913).

*Lamb No. IV.*

1 Put eight ♀s and two ♂s on lamb's back.

2 Temperature: M. 103·2° F.

3 Temperature: M. 101·2° F., E. 103° F.

4 Temperature: M. 102·9° F., E. 102·8° F. Two ♀s and one ♂ fouud attached along back.

5 Temperature: M. 102° F.

6 Temperature: M. 101° F., E. 102° F.

7 Temperature: M. 102·8° F.

8 In the morning the lamb showed weakness; in the evening it bumped into everything. Lamb off its feed. Temperature: M. 102·2° F., E. 102·4° F. Pl. XXII, fig. 6.

9 Lamb could hardly stand, incontinence of urine; in evening was unable to stand unless assisted. One ♀ fell off gorged.

*Autopsy.* Lamb killed at 9 p.m. by bleeding. All organs normal. The coveriugs of the brain were distinctly injected.

*Note.* Blood counts (differential) made before and after paralysis appeared showed no variation from the normal.

The temperatures recorded in the tick-transmissiou experimeuts are quite within the normal range for lambs.

*Attempt to transmit the disease by inoculation* (1913).

*Lamb No. V.*

| Date | |
|---|---|
| 13 v. | Inoculated subcutaneously with 20 c.c. of defibrinated blood taken from Lamb No. IV. |
| 13 v.–1 vi. | Lamb remained perfectly well; no effects from inoculation. |

*Experimental inoculation with mashed-up ticks.*

| | |
|---|---|
| 19 v. | Inoculated in two places along the back with mashed-up ticks: one engorged ♀ from Lamb No. III and one engorged ♀ from Lamb No. IV; three unengorged ♀s from Keremeos. |
| 1 vi. | No bad effects of any sort to date. |

*Experiments with guinea-pigs.*

| | |
|---|---|
| 13 v. | Two guinea-pigs were inoculated along the back with a few drops of fluid from mashed-up ticks. |
| 31 v. | No results of any sort up to date; guinea-pigs destroyed. |
| 14 v. | Six guinea-pigs were inoculated intraperitoneally with 2 c.c. each of defibrinated blood from Lamb No. IV. |
| 14 v. | Six guinea-pigs were inoculated subcutaneously with brain emulsion. |
| 31 v. | No results up to date, when guinea-pigs were destroyed. |

## SUMMARY.

"Tick paralysis" occurs in British Columbia and affects man, sheep, and probably other animals. The disease is caused by the bites of *Dermacentor venustus* Banks. It is usually of short duration, is benign in character, but occasionally it persists for long periods, and may terminate fatally. From an economic point of view, the disease is of some importance to the sheep industry. The causative agent has not been discovered, and the disease has not been reproduced by inoculation. The most likely hypothesis is that the tick injects a toxin which gives rise to symptoms appearing coincidentally with the complete engorgement of the tick. In three consecutive cases, experimentally produced by me in lambs, paralysis occurred six to seven days after the ticks were put on. In no case did I fail to produce paralysis through the agency of the tick bites. It has been proved that *D. venustus* usually bites sheep along the back-bone; possibly the point of attachment may have some bearing on the symptoms or severity of the case. It is probable that other species of ticks may produce a similar disease. No larvae or nymphs were encountered on sheep, and I have no records of their attacking man in British Columbia.

I am indebted to Dr F. Torrance, Veterinary Director-General, for permission to publish this paper.

## Appendix I.

I append the following notes upon the biology of *D. venustus*, since they bear directly upon the subject of this paper, and I have successfully raised the species from the affected locality.

### *Protocols.*

*Lot I* (1912).

| Date | |
|---|---|
| 25 vi. | Gorged larvae of *D. venustus* were collected from a *squirrel* at Merritt, B.C. They were taken to the laboratory at Agassiz, placed in tubes with moistened filter paper, and kept at room temperature. |
| 18 vii. | The first nymph emerged (metamorphosis lasted 23 days). |
| 22 vii. | Six nymphs were placed on a *rabbit* (3–4 days after ecdysis). |
| 27–29 vii. | Three gorged nymphs dropped off the host (two after five days, one after seven days). |
| 29 viii. | One ♂ and two ♀ s emerged (metamorphosis to adult lasted 32 days). |

*Lot II* (1912–1913).

| | |
|---|---|
| 6 vi. | Gorged ♀ s of *D. venustus* were collected by Dr Thomson at Keremeos, B.C., from *horses* imported from the United States. The ticks were forwarded to Agassiz. |
| 12 vii. | The ♀ s began to oviposit (pre-oviposition period eight days). |
| 12 vii. | Larvae emerged (metamorphosis from egg to larva lasted 36 days). |
| 24 ix. | Larvae were placed on a *rabbit* (34 days after ecdysis). |
| 28 ix. | 16 gorged larvae dropped off the host (the period of parasitism lasting four days). |
| 5 xi. | 10 nymphs emerged (metamorphosis from larva to nymph lasted 38 days). |
| 22 xi. | 46 nymphs were placed on a *rabbit* (17 days after ecdysis). |
| 28–30 xi. | Three gorged nymphs dropped off the host (the period of parasitism on the host lasting 7–8–9 days). |
| 20 ii.–4 iii. 1913. | One ♀ and two ♂ s emerged (metamorphosis from nymph to adult lasted 84–94 days in a cold room). |

*Note.* These raising experiments were conducted at room temperature; the temperature varied greatly, according to the weather, and in Lot II the metamorphosis of the adults was no doubt much prolonged.

## Copulation.

The copulation of *Dermacentor venustus* was observed on a lamb's back at Agassiz, British Columbia, on the 24th April, 1913. The act of copulation lasted 33 minutes. The operation began by the male hooking his three posterior pairs of legs under those of the female, then by his rooting about and inserting his hypostome into the vulva. The

palpi were parted as in sucking blood. At times it was noticed that he was moving his mouthparts; later he worked more actively and appeared to be enlarging the opening; he then depressed his capitulum so that his mouthparts were no longer visible. It looked as though he had withdrawn them from the female vulva and had directed them towards his genital aperture where they appeared to be working. A minute or two afterwards his mouthparts were again visible (palpi not parted, nor was the hypostome in the female vulva). A glistening fluid was now seen which increased in amount, and finally surrounded the mouthparts and the vulva. The fluid was later seen to be of a reddish tinge; possibly it originated from the female, as she passed a similar looking material from her anus during the act.

After a time, before the male abandoned the female, he was seen to move up her body so that his hypostome was on a level with hers; the genital orifices would be in juxtaposition. These movements were repeated several times. The reasons for them were made apparent later, when a spermatophore was found attached partly within the vulva. Judging from the movements of the male at one stage of the act, he was exerting pressure with his body to expel the contents of the spermatophore, and when he retreated he felt about with his mouthparts as though to ascertain if the spermatophore was in position. After the male had abandoned the female the fluid surrounding the vulva was gently removed with a brush and a collapsed spermatophore was found attached. The accompanying photograph, fig. 7, shows the spermatophore attached to the vulva.

*Time required for* D. venustus ♀s *to gorge on sheep.*

The average time for the first six females which dropped off gorged was 6·5 days (the shortest period four days, the longest ten days).

*Weights of females before and after gorging on sheep.*

3 ♀s unengorged: average weight = 0·001 gramme.
1 ♀ (gorged) weighed 0·610 gramme.
1 ♀ „ „ 0·550 „
1 ♀ „ „ 0·600 „
Average = 0·587 „

*Pre-oviposition period* lasted eight days at summer heat and four, five and six days in the case of three ♀s with intermittent heat in incubator at 32° C.

*Oviposition* (1912).

15 VII. 51 eggs laid.

15–16 VII. 479 eggs laid.

From 12.30 noon to 3.20 p.m., 66 eggs were laid, which equals 2·5 minutes for each egg. From 5.15 p.m., to 12 noon on the following day a female laid 474 eggs, which equals 2·3 minutes for each egg.

*Longevity.*

A partly gorged female taken from a lamb on 5 VI. 1913 was still alive on 24 VII. 1913 (a period of 49 days). A fully gorged female which dropped off a lamb on 25 IV. 1913 was still alive on 24 VII. 1913 (a period of 89 days).

Two unengorged females were alive and active after 50 days, and attached themselves to two different animals.

## Summary.

*Duration of parasitism upon the host.*

The larvae remain upon the host four days, nymphs 4–9 days, females 4–10 days (average for six ♀s = 6·5 days). The time required for metamorphosis from egg to larva = 36 days, at summer temperature; gorged larva to nymph = 24–38 days at summer heat; gorged nymph to adult = 32 days (in summer) to 84–94 days in a cold room in winter.

The pre-oviposition period lasted eight days in summer and four, five and six days in the case of three ♀s with intermittent heat in an incubator at 32° C.

The process of copulation, observed on a lamb's back, lasted 33 minutes. The males copulate with semi-gorged females.

*Increase in weight of ♀s through feeding.*

Three unfed ♀s averaged 0·001 gramme; three replete ♀s averaged 0·587 gramme in weight.

*Oviposition:* A ♀ was observed to oviposit as follows:

Beginning on 15 VII. 1912, the first day, she laid 51 eggs; first to second 479 eggs (66 eggs or 2·5 minutes per egg laid between 12.30 p.m. to 3.20 p.m.); from 5.15 p.m. on second day to 12 noon on third day she laid 474 eggs, or 2·3 minutes for each egg.

## Appendix II.

Todd (1912) addressed letters to a number of doctors in British Columbia, asking for information concerning the ill-effects following tick bites. He records that six letters were received, mentioning instances in which tick bites were followed by paralysis, and occasionally by death. I would cite the following:

"Dr G. B. H., Creston, B.C.: A little girl, four years of age, gradually lost the use of her legs during two or three days until she was unable to stand. A tick was removed from the nape of the neck and within three days the child was well again."

"Dr O. M., Vernon, B.C., January 1912: A healthy child, three-and-a-half years of age, had been perfectly well until two hours before examination. When the patient was seen there was no temperature and the pulse was normal, but the legs were almost completely paralysed. The child could not stand, and the reflexes were gone. A tick was found firmly attached to the base of the neck; it was removed. The paralysis continued during the day. Next morning there was slight improvement, and by the evening the child had recovered the use of her legs.

Dr N. knows of an instance in which an adult complained of weakness of the legs after a tick bite on the back."

"Dr W. O. R., Nelson, B.C.: About 1900 a child died suddenly with symptoms of acute ascending paralysis. After death a large tick was found at the nape of the neck.

In 1901, a second child with the same symptoms died after an illness of two days. A tick was found attached to the right temple.

The knowledge of these two cases suggested the presence of a tick when a third child, previously very healthy, was seen, whose legs had been becoming weaker for two days. One tick was found at the nape of the neck; it was removed, and in two days the child was quite well again.

On April 10th, 1912, a little girl, three years of age, had become paralysed. The legs were completely paralysed and the reflexes gone; paresis of the arms was marked. Three ticks were removed from the nape of the neck and the child recovered completely."

Dr Todd adds: "These notes are made public in the hope that they may induce physicians who have seen or who may see similar cases to publish their experiences, for it seems possible that an undescribed

disease, caused by ticks, may occur in British Columbia. The subject demands investigation."

The "ticks" referred to in Dr Todd's paper are most probably *D. venustus* as this species is common in the districts he mentions.

Nuttall (1911) in his notes on the biology of *Ixodes*, refers to *Ixodes pilosus*.

I quote the following:

"*Relation to Disease.* According to C. W. Mally (IX 1904), Cape of Good Hope, the farmers around Carlisle Bridge have no doubt but that this tick produces 'paralysis' in sheep, especially in merinos.... The tick is, however, frequently found on healthy sheep."

Eaton (1913) reports a case of tick bite, followed by widespread transitory muscular paralysis, in a little girl.

Briefly, the symptoms he describes are as follows:

A little girl, four years of age, became restless and unsteady on her feet. She also lost her appetite. When she was undressed and put to bed a tick was found attached to the back of the right shoulder. The tick was probably an *Ixodes* (sp. ?).

On the second day the child could not stand, and appeared very ill and in a state bordering on delirium (temperature 101·4° F.). The muscles of the lower limbs were motionless, the arm muscles could be moved, the knee jerks were absent. Over the lower part of the right scapula was a pink patch about the size of a penny, in the centre of which was a purplish-black spot a quarter-of-an-inch in diameter. In the middle of this was an aperture in which lay the head-parts of the tick (the body had been cut off); these were scraped out and the cavity cleansed with pure carbolic.

On the third day there was no sign of return of voluntary movement in the legs, but the muscles were not so limp. On the fourth day the knee-jerks were obtainable but with difficulty; the child could now stand and even walk a few steps with support. The pupils of the eyes were still inactive to light. Diarrhoea present from third day. On the fifth day the child could walk without support.

Eaton quotes other cases noted by Cleland. Cleland refers to a statement of Bancroft's that the bites of Queensland ticks (sp. ?) frequently kill dogs and cats. One attack is said to confer immunity.

BRITISH MUSEUM
NATURAL HISTORY

Fig. 1.

Fig. 2.

Fig. 3.

*S. Hadwen, phot.*

Fig. 4.

BRITISH MUSEUM
NATURAL HISTORY
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D

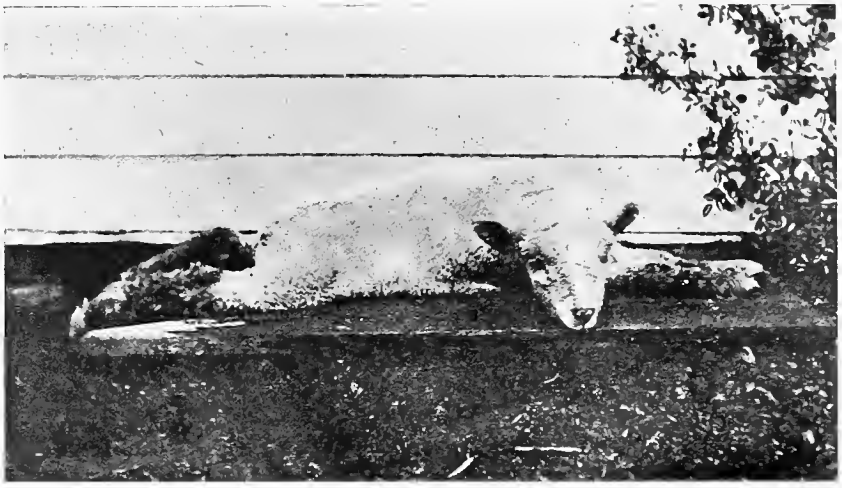

Fig. 5.

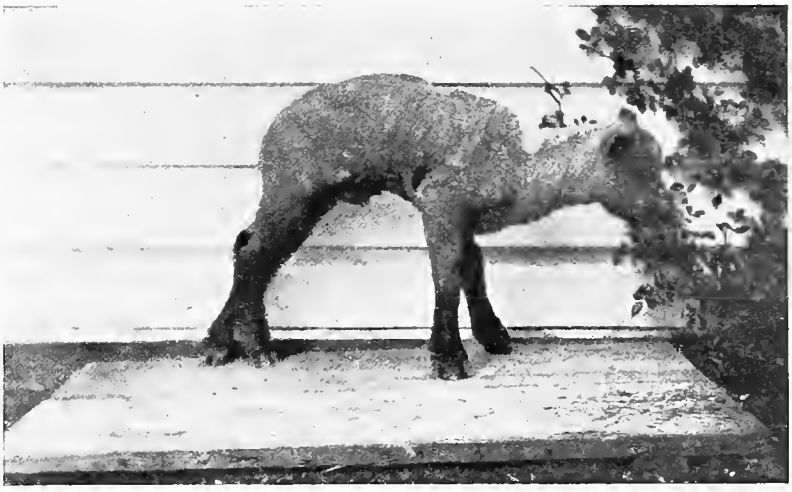

Fig. 6.

Fig. 7. Fig. 8.

*S. Hadwen, phot.*

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## EXPLANATION OF PLATES XXI AND XXII.

(Photographs by S. Hadwen.)

Fig. 1. Effect of bites under the skin (Lamb No. I).

Fig. 2. Eleven *D. venustus* attached to back of Lamb No. I.

Fig. 3. Lamb No. IV. Two females and one male attached. Line drawn on lamb's back to indicate median line.

Fig. 4. Enlargement of lower ticks in Fig. 3, showing position of male and female.

Fig. 5. Lamb No. III, showing paralysis.

Fig. 6. Lamb No. IV, showing loss of coordination.

Fig. 7. *D. venustus* ♀ (gorged), with spermatophore attached.

Fig. 8. *D. venustus* ♀ (unengorged).

# EXPERIMENTAL "TICK PARALYSIS" IN THE DOG.

By SEYMOUR HADWEN, D.V.Sci.,
and G. H. F. NUTTALL, F.R.S.

(*From the Quick Laboratory, University of Cambridge.*)

The following note is published by way of supplement to the foregoing paper on "Tick Paralysis" by Hadwen, as it records a positive experiment carried out in Cambridge upon a dog experimentally infested with *Dermacentor venustus* obtained from Canada.

The ticks (4 ♀s and 1 ♂) were collected about 5. vi. 1913 by Mr Stanford from his own person whilst prospecting in the mountains near Nelson, British Columbia.

## *"Tick Paralysis" in the Dog.*

The dog upon which the following experiment was carried out had been under observation for close on 14 months at the Field Laboratories, Cambridge, having previously been used for experimental observations on piroplasmosis. On 17. v. 1912 it was inoculated subcutaneously with blood containing *Piroplasma canis*; on 25. v. a few parasites were present in the dog's blood and on the following day it was treated in the usual way by the subcutaneous injection of 10 c.c. of 2% aqueous solution of Trypanblue. The animal promptly recovered from piroplasmosis and remained perfectly healthy up to 8. vii. 1913, the date upon which our experiment was started, as recorded in the protocol which follows:

| Date | Day | Temperature °F. a.m. | Temperature °F. p.m. | |
|---|---|---|---|---|
| 8. VII. 13 | 1 | A single *D. venustus* ♀ was placed upon the dog. | | |
| | 2 | 100·6 | — | The tick attached itself on the right side of the neck, just in front of a line joining the scapular angles |
| | 3 | 101·6 | 101·4 | |
| | 4 | 102 | 101 | |
| | 5 | 101·4 | 100·2 | |
| | 6 | 99·4 | 100 | |
| | 7 | 100·8 | 101 | |
| | 8 | 101 | 101·2 | |
| | 9 | 101·2 | 101·2 | Symptoms of paralysis appeared, hind quarters affected; dog very restless and falling about when attempting to walk. Front legs fairly under control. Sensation in hind legs unimpaired but movements incoordinate. |
| | 10 | 100 | 101 | Front limbs affected; dog could only raise his head a little, incontinence of urine. Loss of appetite. |
| | 11 | 100 | 101 | Dog looking very ill, breathing laboured, had to be fed, paralysis persisting. |
| | 12 | 101 | 99·8 | |
| | 13 | 99·2 | 99·2 | |
| | 14 | 99·2 | 99·6 | Symptoms subsiding. |
| | 15 | 100·6 | — | Almost well. |
| | 16 | — | — | Dog as well as ever, can run and jump over obstacles. |
| | 18 | Tick removed, about $\frac{2}{3}$ gorged. | | |

This experiment demonstrates conclusively that a single tick is capable of producing "Tick Paralysis" in the dog.

### *Negative results with a Horse and Jackal.*

On 25. VII. 1913, two *D. venustus* ♀s, which were all that survived of the ticks collected 5. VI. 1913, by Mr Stanford, were placed upon a jackal and a horse respectively after the ticks had been placed overnight at 30° C. Both ticks attached themselves to their hosts, they did not gorge to repletion and produced no effect.

### *Deaths in Guinea-pigs to which* D. venustus *were applied.*

On 22. VII. 1912, one of us (G. H. F. N.) received some eggs of *D. venustus* from Dr C. G. Hewitt, who had received the parent ticks from Washington, D.C. The ticks which issued from the eggs were placed on various hosts, with the object of raising them to maturity and studying their biology.

*Experiment I.* (Negative.)

| | |
|---|---|
| 26. VII. 1912 | *D. venustus* larvae were placed on a guinea-pig. |
| 29. VII.–1. VIII. 12 | Larvae dropped off gorged. |
| 8. VIII. 12 | Nymphs began to emerge. |
| 13. IX. 12 | Nymphs placed on a guinea-pig. |
| 18–23. IX. 12 | Nymphs dropped off gorged. |
| 2. X. 12 | Adults emerged. |
| 24. XI. 12 | Adults (4 ♂ s, 4 ♀ s) placed on ram's scrotum. |
| 9. XII. 12 | 1 ♀ dropped off gorged. |

*Experiment II.*

| | |
|---|---|
| 6. VIII. 1912 | *D. venustus* larvae were placed on a guinea-pig. |
| 9–12. VIII. 12 | Larvae dropped off gorged. |
| 20. VIII. 12 | Nymphs began to emerge. |
| 19. X. 12 | Nymphs placed on a guinea-pig. |
| 24. X. 12 | *Guinea-pig died* on the 6th day after the nymphs were applied. |

*Experiment III.*

| | | |
|---|---|---|
| | 7. IV. 1913 | *D. venustus* larvae were placed on a guinea-pig. |
| | 9–10. IV. 13 | Larvae dropped off gorged. |
| | 11. IV. 13 | *Guinea-pig died* on 5th day after the ticks were applied. |
| | 17. IV. 13 | Nymphs began to emerge. |
| *a.* | 13. V. 13 | Nymphs placed on a guinea-pig. |
| *a.* | 19. V. 13 | *Guinea-pig died* on 7th day after ticks were applied. |
| *b.* | 2. VI. 13 | Nymphs placed on a ram's scrotum. |
| *b.* | 8–13. VI. 13 | Nymphs dropped off gorged. |
| *b.* | 23. VI. 13 | Adults began to emerge. |
| | 10. VII. 13 | Adults (3 ♀ s, 3 ♂ s) placed on ram's scrotum. |
| | 18. VII. 13 | 1 ♀ dropped off gorged. |

## *Conclusions.*

The condition of "Tick Paralysis" experimentally produced in a dog in Cambridge through the application a single *Dermacentor venustus* ♀ from Canada is the same as that observed in sheep, as described in the preceding paper. The examination of the dog's blood proved negative.

The negative results of inoculations and the absence of fever indicate that the disease is not infective, although the incubation period suggests the contrary. On the hypothesis that the symptoms are due to *toxins* given off by the tick, the "period of incubation" might be explained on the supposition that it is only when the tick commences to engorge or feed rapidly, some days after it has become attached, that it gives off the hypothetical toxin in its saliva in sufficient quantity to produce pathogenic effects. Where the ticks are picked or rubbed off early no

pathogenic effects follow. Under natural conditions, freshly attached ticks are frequently removed from their seats of attachment to man and animals, consequently many subjects that have been bitten do not suffer from serious after-effects.

On the hypothesis that the disease is *infective*, the negative results which follow the premature removal of the ticks may be explained on the assumption that the hypothetical parasite undergoes a development in the tick whilst the latter is attached to the host, and that the parasite only enters the vertebrate host after the tick has remained attached to this host for some days. This supposition is strengthened by the observations of Nuttall and Hindle upon East Coast Fever, which are published in this number of *Parasitology*, wherein it is demonstrated that the tick *Rhipicephalus appendiculatus* is non-infective during the first two or three days of its attachment to susceptible cattle.

We hope, in the course of further investigations, to throw more light upon this interesting affection, and to determine if it is due to an infective process or not.

We can offer no explanation of the deaths observed in guinea-pigs following the application of *D. venustus* larvae and nymphs (Washington strain); they were certainly attributable to the ticks although but few of these were placed upon the animals. The examination of the guinea-pigs at autopsy proved negative. One of us (S. H.) has not observed any ill effects in rabbits upon which *D. venustus* larvae and nymphs were raised in Canada. As far as our records go it is only the adult ticks which attack man, or larger animals, in Canada.

# THE HERTER LECTURES.

## III[1]. *PIROPLASMOSIS.*

By GEORGE H. F. NUTTALL, M.D., Ph.D., Sc.D., F.R.S.
*Formerly Associate in Hygiene, Johns Hopkins University;*
*Fellow of Magdalene College, Quick Professor of Biology*
*in the University of Cambridge.*

(With 14 Text-figures.)

The diseases included under the general term of piroplasmosis are amongst the most devastating which affect domesticated animals, and they are, consequently, of great economic importance. As far as known, all forms of piroplasmosis are tick-transmitted.

### *Diseases due to Piroplasma (= Babesia).*

Strictly interpreted, the term "piroplasmosis" applies to diseases caused by intracorpuscular parasites belonging to the genus *Piroplasma* (= *Babesia*[2]) which possess certain definite characters, produce definite symptoms, and are communicable by blood inoculation.

The striking features which characterise *Piroplasma* are the following: on examining the infected blood corpuscles and enumerating them in accordance with the types of parasites they contain, it will

[1] Owing to the lecture not having been written until after it was delivered in the Medical Department of the Johns Hopkins University, 10 October, 1913, the author has taken the opportunity of adding a considerable amount of new matter and has somewhat altered the original form of the lecture. 30. vi. '13. G. H. F. N.

Figs. 1, 3, 4, 9, 10 and 14 are reprinted from *Parasitology*, vols. i.–v.; the remaining 8 figures (2, 5–8, 11–13) have not hitherto been published. All except Fig. 9 are by the author.

[2] The generic name *Babesia* has priority, and is coming into general use, although the name *Piroplasma* is more usually employed by American and British writers.

be found that 30 to 40 % contain *two piriform parasites joined at their tapering extremities*—this appearance is characteristic of what I regard as the genus *Piroplasma*, of which the species *canis, caballi, ovis, bovis* or *bigeminum*, and *divergens* may be taken as examples. Of the remaining infected corpuscles about 50–70 % contain single parasites of varying form, whilst a few (anywhere from 0·5 to 11 %) show characteristic dividing forms, whose significance I pointed out some years ago, with Graham-Smith. *Piroplasma canis* and *P. pitheci* differ slightly from other piroplasms in that some infected corpuscles (about 1–4 %) contain more than two parasites (*i.e.* 4–16) although in *P. bovis* four parasites are occasionally found within a corpuscle. The usual mode of multiplication of *Piroplasma* is shown in the accompanying figure (Fig. 1).

True piroplasmosis occurs in cattle, sheep, horses and dogs in many parts of the world. The parasites which cause the disease in each of these species of mammals are specific in respect to their pathogenic action: for instance, the parasite which occurs in the dog (*P. canis*) is only capable of setting up the disease in the dog. Parasites having the morphological characters of *Piroplasma* have been discovered in the monkey and rat (*P. pitheci, P. muris*), but the symptoms they produce and their mode of transmission are unknown.

In the domesticated animals above enumerated, the presence of piroplasms in the blood is accompanied by a definite train of symptoms following upon the animals being attacked by pathogenic ticks. Usually about 8–10 days after the animals have been attacked by the ticks they show high fever, loss of appetite, haemoglobinuria, icterus, and a large number of the animals die in a few days—anywhere from 25–100 % succumbing to the infection. The haemoglobinuria is chiefly due to the destruction of the blood corpuscles by the parasites, the haemoglobin being eliminated from the kidneys. The urine may appear lightly tinged with haemoglobin or very dark, depending upon the intensity of the blood destruction. In severe cases, the number of corpuscles in the circulating blood may be reduced to a third or less, the blood, consequently, appearing thin and watery. When animals "recover" they do so slowly, and the parasites grow very scarce in the blood so that they cannot, as a rule, be detected microscopically.

The parasites persist in the blood of "recovered" animals for years after they have, to all outward appearances, resumed a healthy condition. "Recovered" or "salted" animals are not susceptible to reinfection, and consequently possess enhanced value in countries where piroplasmosis is

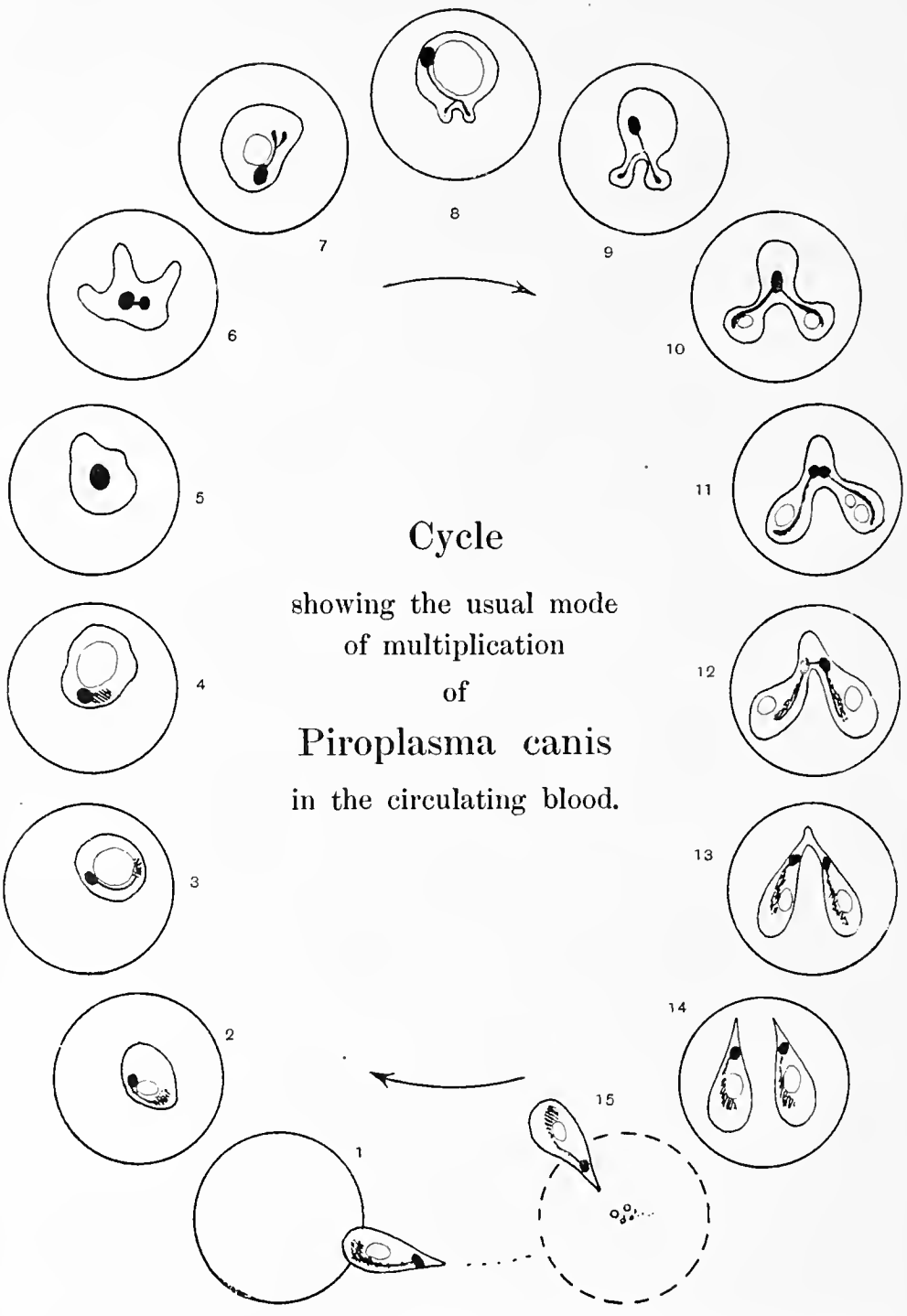


Fig. 1. *Piroplasma canis:* (1) a free piriform parasite which has just left a blood corpuscle enters a normal corpuscle and (2–4) assumes a rounded form, remaining quiescent for a time after which it grows in size. It then becomes actively amoeboid (5, 6), and again becomes rounded (7). Two symmetrical processes (8–10) are then protruded, which rapidly enlarge at the expense of the body of the parasite. Each of these processes (11–13) gives rise to a mature piriform parasite, which remains attached to its fellow by a thin strand of protoplasm. The parasites next become separated (14) and, by active swimming movements, burst out of the corpuscle (15) whose haemoglobin escapes into the plasma. The free parasite immediately re-enters a fresh corpuscle. (Nuttall and Graham-Smith, 1907.)

endemic. The blood of "salted" dogs and cattle has been found to be infective for three to eight years after the acute attack has subsided. In nature, piroplasmosis is transmitted only by ticks which have previously fed upon infected animals either in the acute stage of the disease or in the "salted" condition. It is owing to indigenous animals being "salted" in regions where piroplasmosis is endemic that the ticks of the region continue to harbour the parasite and constitute a potential source of danger to freshly imported animals coming from places where the disease is absent.

*Bovine Piroplasmosis.*

Bovine piroplasmosis is due to at least two species of *Piroplasma*: *P. bovis* and *P. divergens*.

The latter parasite (Fig. 2) judging from specimens which have reached me hitherto is confined to Europe, and in nature is transmitted

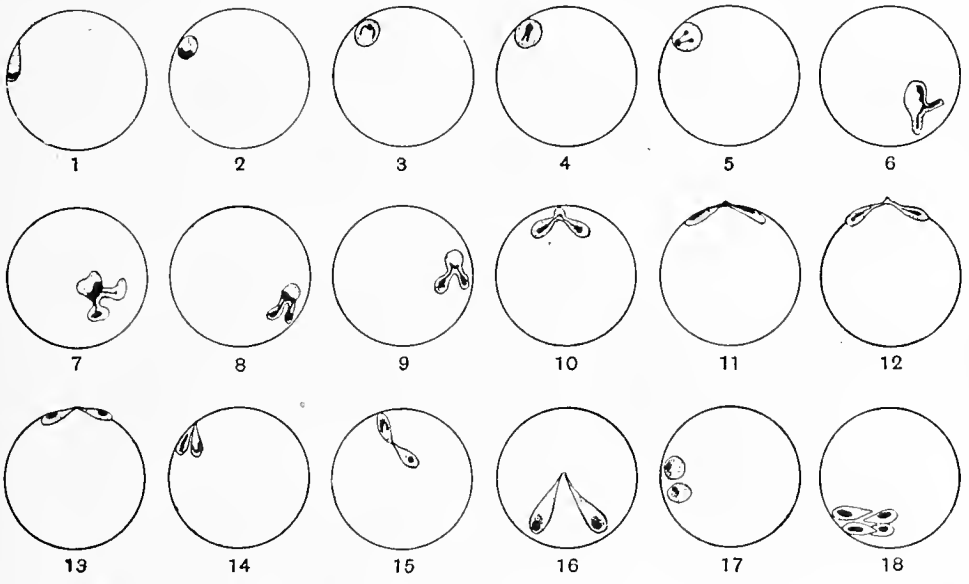


Fig. 2. *Piroplasma divergens:* progressive stages of development corresponding to those observed in *P. bovis* (q.v.); the parasite is, however, much smaller than *P. bovis*, although occasionally fairly large piriforms (16) may be encountered. (Original, G. H. F. N. del.)

by *Ixodes ricinus*[1], the common European cattle tick. I have received specimens of *P. divergens* and *I. ricinus* from cattle suffering from "redwater" in Norway, Germany, Russia, Hungary, Great Britain, and Ireland, and I know that this form of redwater occurs in Finland, Sweden

[1] See Description in *Ticks*, Part II, pp. 143, 293, 334 and *Parasitology*, VI. p. 91.

and France where *I. ricinus* is prevalent. The transmission of the disease by means of larval stages of *I. ricinus*, the progeny of ticks collected from diseased cattle suffering from piroplasmosis in Germany, England and Ireland, has been demonstrated experimentally by Kossel and his colleagues, by Stockman, and by myself respectively. Although the symptoms due to *P. divergens* infection are similar to those produced by *P. bovis*, the disease appears to be milder. Animals which have "recovered" from *P. divergens* infection are susceptible to infection with

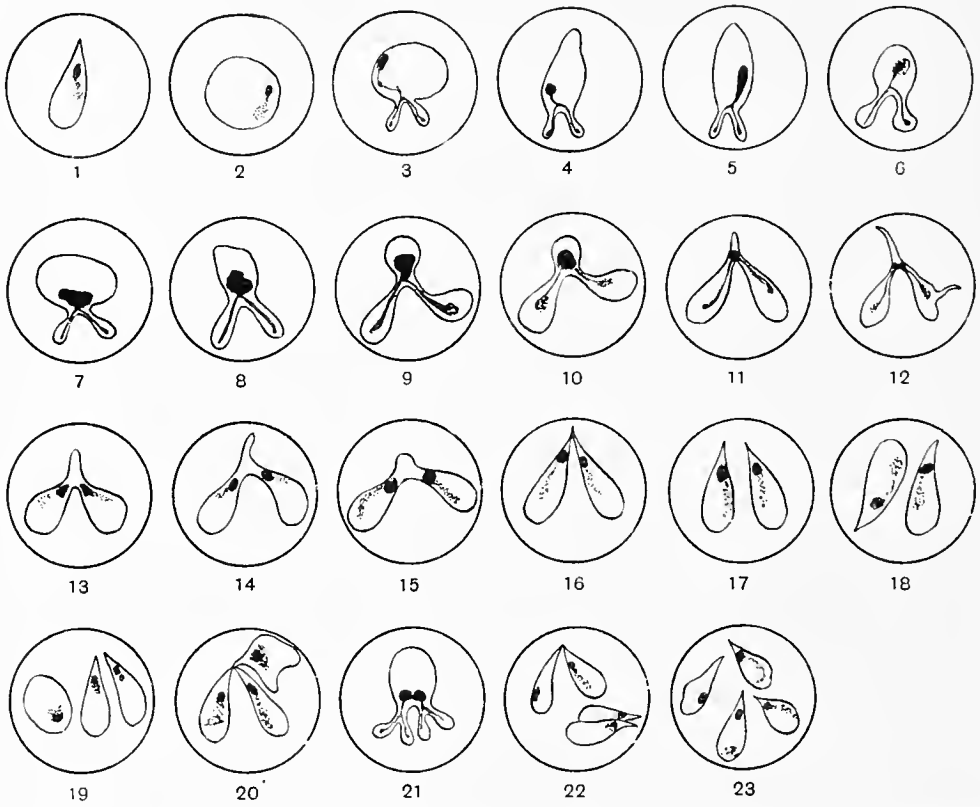


Fig. 3. *Piroplasma bovis*, showing the same mode of multiplication as *P. canis*; (21) a parasite dividing directly into four. (Nuttall and Graham-Smith, 1907.)

*P. bovis*. The parasites, moreover, show distinct morphological differences which were recognized by Kossel and others, although it is but recently that MacFadyean and Stockman gave *P. divergens* its distinctive name.

The well-known Texas fever of the United States, generally known as redwater or bovine haemoglobinuria in other parts of the world, is due to *P. bovis* (Fig. 3), and appears to be transmitted in nature almost solely by *Boophilus annulatus* and its varieties, *B. australis* and

*B. decoloratus.* *Piroplasma bovis* is widely distributed in warm countries and the tropics—it occurs in N. and S. America, Australia, Asia and Africa, and occasionally in Southern Europe. Redwater has been repeatedly reproduced experimentally by means of larval *Boophilus*, the progeny of ticks which have sucked the blood of animals suffering from redwater. This form of piroplasmosis has been the cause of great financial losses, it having been estimated that in the United States alone the annual loss has amounted to forty million dollars.

### *Equine Piroplasmosis.*

Redwater, or "biliary fever" in horses, as I have shown with Strickland, is due to two parasites which are morphologically distinct: *Nuttallia equi* (Laveran), of which I shall speak presently, and *Piroplasma caballi* Nuttall (Fig. 4), the cause of true piroplasmosis in equines. When

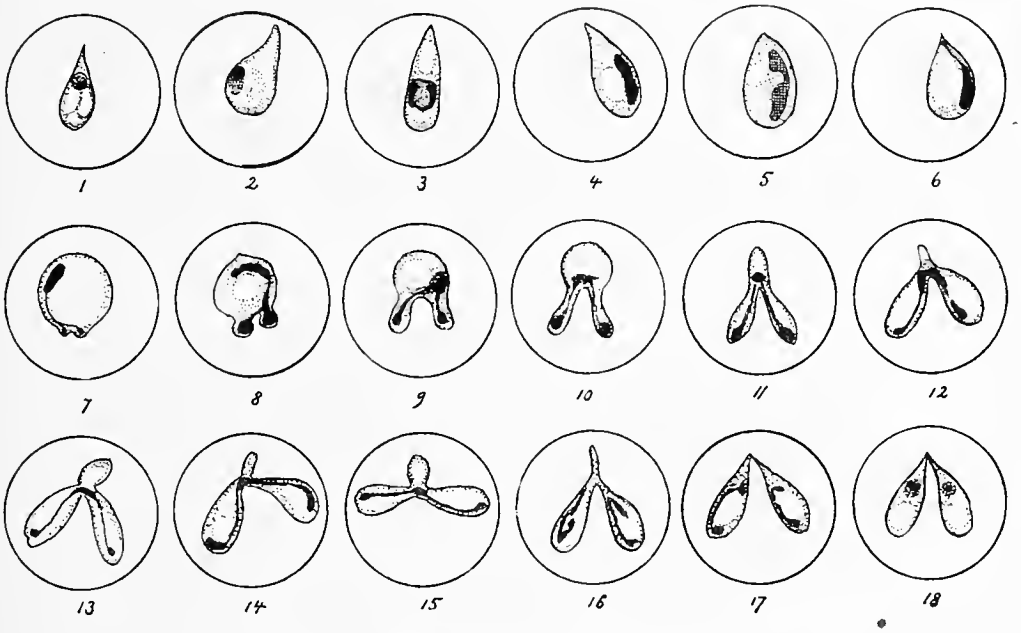


Fig. 4. *Piroplasma caballi*, showing the same mode of multiplication as *P. canis*. (Nuttall and Strickland, 1912.)

a horse "recovers" from *P. caballi* infection it is still susceptible to *N. equi* infection. *Piroplasma caballi* occurs in Russia, Roumania, in Transcaucasia, and apparently extends across Siberia. *Dermacentor reticulatus* has been shown by experiment to transmit the disease, and this tick is distributed over the geographical area mentioned, besides occurring in Western Europe, including Great Britain, where

it is, however, relatively rare. Although I have received blood-films from horses suffering from biliary fever in other parts of the world, none of the films contained parasites similar to *P. caballi*[1].

### *Ovine Piroplasmosis.*

True piroplasmosis, due to *P. ovis*, has been observed in sheep in Italy, Turkey, Roumania and Transcaucasia. I have been informed that it has recently been discovered in Egypt[2]. The recorded presence of ovine piroplasmosis in S. Africa and N. America is due to errors of observation. It has been demonstrated by experiment that *Rhipicephalus bursa* conveys the disease. The geographical distribution of this tick, judging from specimens which have reached me, appears to be limited chiefly to Southern Europe, the Islands in the Mediterranean, Transcaucasia, and North Africa, although Neumann states that it occurs both in E. and W. Africa as far as the Cape and also in the West Indies. I have only seen *P. ovis* in blood-films from Roumania and Italy.

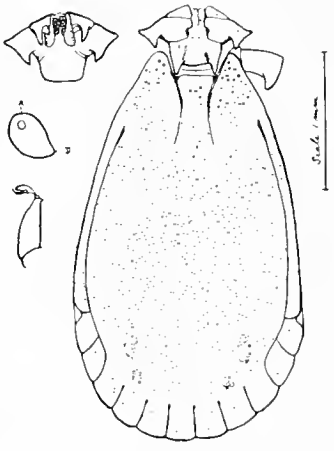

Fig. 5.

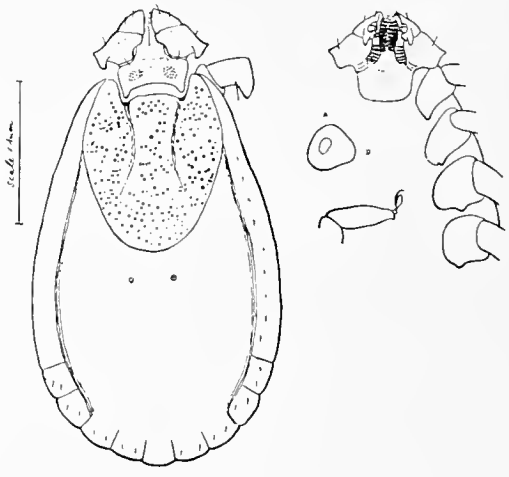

Fig. 6.

Fig. 5. *Haemaphysalis leachi* ♂. (Original, G. H. F. N. del.)
Fig. 6. ,, ,, ♀. ,, ,,

[1] On 17 January, 1913, Dr S. T. Darling, Chief of the Department of Sanitation, Ancon, Panama, sent me a blood-film showing *P. caballi* taken from a horse in Panama. The ticks he sent us for determination and which were taken from the horse were *Dermacentor nitens* and *Amblyomma cajennense*—it is probable that the former is the carrier, since it is usually found on horses.

[2] In blood-films I have received (5. VI. '13) from Mr F. E. Mason of Cairo, we have only been able to detect a few small single intracorpuscular parasites, none of which were typical of true piroplasms.

*Canine Piroplasmosis.*

Two forms of piroplasmosis have been observed in dogs, the commoner and more widely distributed being due to *P. canis*. In India, *P. canis* (Fig. 1) is transmitted by *Rhipicephalus sanguineus*, a tick which has accompanied the dog practically all over the world. This tick is the

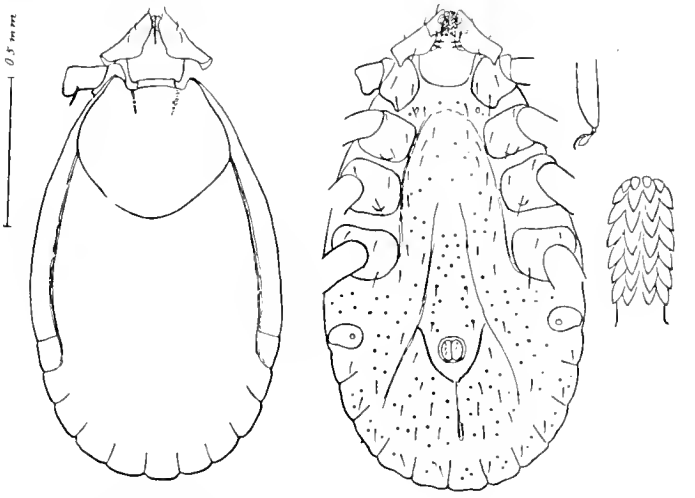


Fig. 7. *Haemaphysalis leachi* nymph. (Original, G. H. F. N. del.)

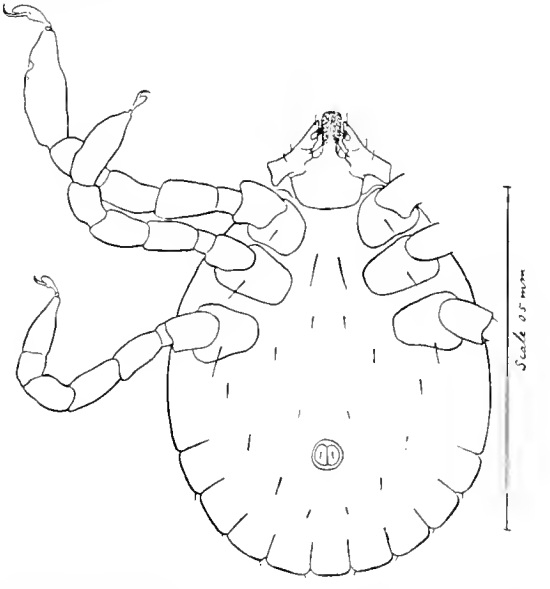


Fig. 8. *Haemaphysalis leachi* larva. (Original, G. H. F. N. del.)

probable vector of *P. canis* throughout Asia, Southern Europe and North Africa. *Haemaphysalis leachi* (Figs. 5–8) is the vector over the greater part of Africa from Cape Colony northwards. Attempts to

infect the fox and jackal with the parasite have been attended by failure.

On the other hand, another parasite (*P. gibsoni* Patton), which is somewhat different to *P. canis* morphologically, is stated to produce infection both in dogs and jackals in India.

*Concerning the Ticks which transmit Piroplasma.*

As I have already stated, piroplasmosis is conveyed to cattle by *Boophilus* and *Ixodes ricinus*, to sheep by *Rhipicephalus bursa*, to dogs by *Rhipicephalus sanguineus* and *Haemaphysalis leachi*, and to horses by *Dermacentor reticulatus*. A number of other species of ticks may, no doubt, play a part as carriers, but the ones I have named are the chief vectors, and in each case they have been proved to transmit piroplasmosis under experimental conditions.

Since piroplasmosis only occurs in the presence of ticks, it is now generally recognized that to grapple with the problem of prevention a knowledge of their life habits is essential. In the time at my disposal I can only refer briefly to the subject. All Ixodid ticks lay eggs whence they emerge as hexapod larvae. As soon as their chitinous exoskeleton has hardened sufficiently, the larvae may feed upon a vertebrate host. After feeding, they remain quiescent for a period, after which they moult and emerge as nymphs. These feed again, undergo metamorphosis, and finally emerge as adult males or females. In the case of *Boophilus* we have a one-host tick, for it remains, as a rule, upon the host from the larval to the adult stage, moulting twice whilst remaining upon the host. In *R. bursa* we have a two-host tick, since it remains upon the host during its larval and nymphal stages, moulting once upon the host: it abandons the host as a fully-fed nymph, undergoes its metamorphosis upon the ground, and emerges as an adult, which has to seek a second host. All of the remaining pathogenic species of ticks above enumerated are three-host ticks, for they drop from the host when replete both in the larval and nymphal stages to undergo their metamorphosis upon the ground; in other words, the larva, nymph and adult have each to seek a host. In all of the ticks here mentioned the sexes copulate upon the host, and the replete fertilized females drop to the ground where they seek shelter and oviposit. The number of eggs laid varies with the species and individual. Taking average figures from my raising notes:

| | | | |
|---|---|---|---|
| *Boophilus decoloratus* | lays | 1200–4500 | eggs. |
| *Ixodes ricinus* | „ | 2400–3200 | „ |
| *Rhipicephalus bursa* | „ | 4900–6900 | „ |
| *Rhipicephalus sanguineus* | „ ca | 2000–3000 | „ |
| *Haemaphysalis leachi* | „ | 2400–4800 | „ |

The rate of metamorphosis varies considerably according to the temperature at which the ticks are maintained. In *Boophilus*, the life-cycle is rapidly completed because the tick is incubated upon the warm blooded host throughout its parasitic period, and it does not lose time in having to find a host after each ecdysis. In *Boophilus*, the cycle from egg to egg may last 120 days, in *R. bursa* it lasts somewhat longer, in *I. ricinus* it lasts 178 days, these being minimum periods observed under experimental conditions.

### *Behaviour of Piroplasma in Ticks.*

When a fertilized female tick has fed upon an infected animal the parasites she imbibes undergo development within her body. About the fourth or fifth day after the parasites have entered the tick's gut, there appear free club-shaped bodies which move about with vermiform movements and are encountered in the gonads. Their further development is obscure, but in infective ticks both Christophers (Fig. 8) and Marzinowsky state that they have found minute bodies, which may provisionally be called Sporozoites, in the salivary glands.

In *Boophilus* and *Ixodes* the larvae, descended from infected parents, are infective. In *H. leachi* the larvae and nymphs are not infective; the ticks, descended from an infected parent, only infect the host when they have attained maturity (Lounsbury, Nuttall). In *R. sanguineus* the larvae are not infective, but the nymphs and adults are; when nymphs are fed on an infected host the adult tick is infective (Christophers). With *R. bursa* (two-host tick) the larvae are not infective, but the adults are (Motas). I have found adults of *H. leachi* infective after starving for seven months, and believe that ticks harbouring *Piroplasma* remain infective as long as they live.

Unfed ticks may withstand prolonged starvation. I have seen unfed larvae of *I. ricinus* still lively after 176 days starvation; *B. annulatus* larvae were lively after 251 days starvation; *R. bursa* adults were lively after 343 days starvation. In this connection, I only mention the longevity of stages which may transmit piroplasmosis. It is clear that three-host ticks are the most difficult to eradicate by treating infested

animals with tick-destroying dips or by removing the hosts from infested pastures for prolonged periods of time with the object of starving the ticks.

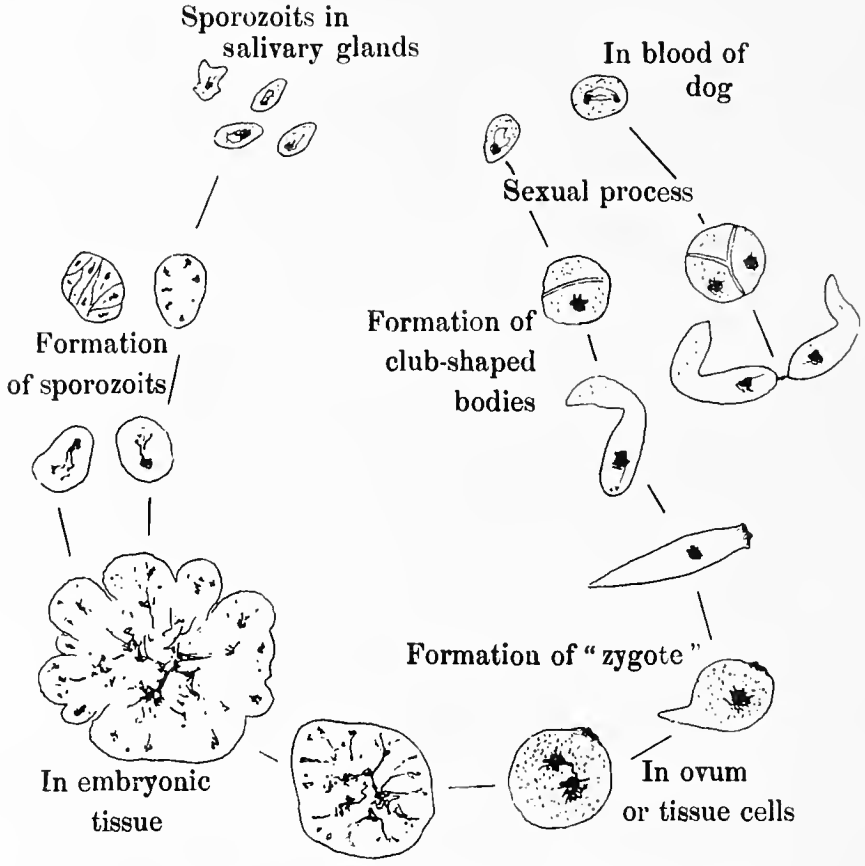


Fig. 9. *Piroplasma canis*, showing the developmental cycle in *Rhipicephalus sanguineus* according to Christophers (1912).

*Diseases due to Nuttallia.*

The first parasite belonging to this genus to be discovered was the one occurring in the horse to which Laveran (1901) gave the name of *Piroplasma equi*. As the morphology of this parasite does not agree with that of a true *Piroplasma*, as defined by me, França (1909) placed it in another genus which he named *Nuttallia*. That he was justified in taking this step is clear from subsequent observations carried out upon the parasite by myself and Strickland (1910–1912, Fig. 10) in the course of which we clearly differentiated it from *P. caballi*. Through the courtesy of Prof. E. J. Marzinowsky, of Moscow, Russia, I have been able to examine blood-films showing so-called piroplasms in deer and reindeer, and I can also refer these to the genus *Nuttallia*.

*Nuttallia equi* appears to be much more widely distributed than *Piroplasma caballi* (q.v.), it produces a similar disease with haemoglobinuria and jaundice, etc. in Italy, Sardinia, in many parts of Africa, Transcaucasia, India, Southern Annam and Brazil, from all of which countries blood-films for examination have been sent to me by correspondents.

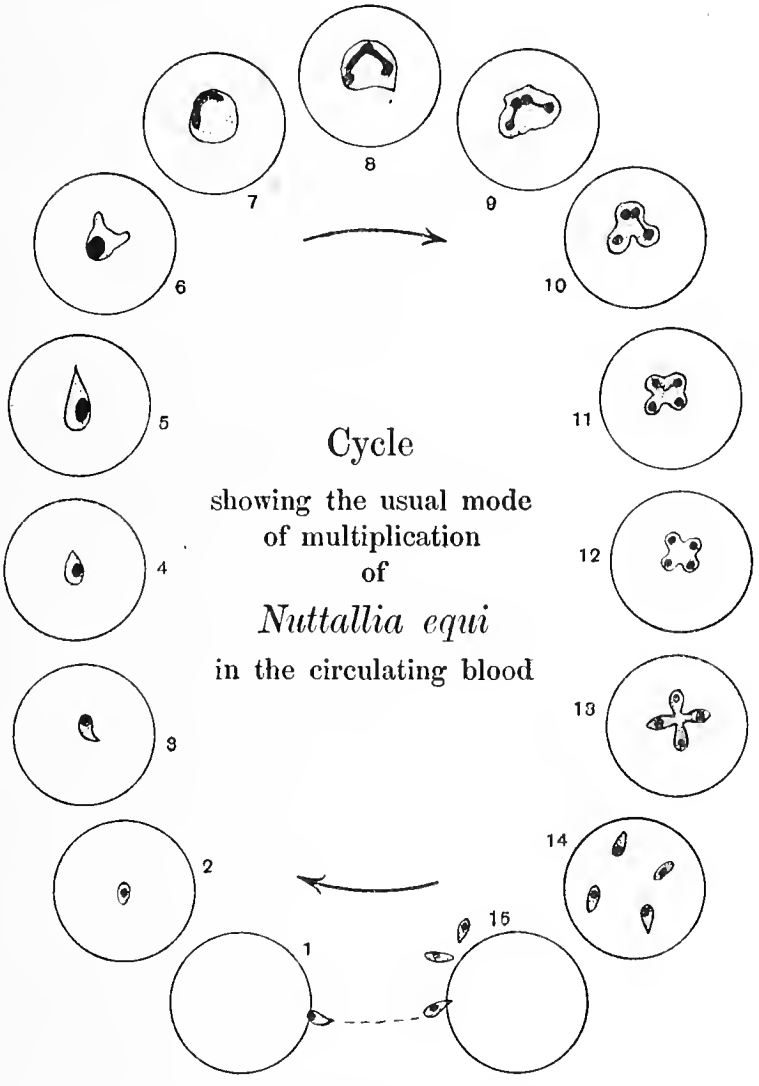


Fig. 10. *Nuttallia equi:* (1) entrance of minute oat-shaped parasite into a corpuscle; (2–5) the parasite grows in size whilst continually but slowly altering its shape; (6) larger parasite which is actively amoeboid; (7–10) successive stages of division of the chromatin; (11–15) process of division repeatedly observed in living parasites: the formation and breaking-up of the cross-forms, the scattering of the daughter-cells within the corpuscle, and their escape from the corpuscle. (Nuttall and Strickland, 1912.)

Our experiments showed that a horse after recovery from *P. caballi* infection succumbed to the inoculation of *N. equi* blood. Conjoined pairs of piriform parasites are never observable in *N. equi* infection, about 90 % of the infected corpuscles contain single parasites of all shapes and sizes, many of them being much smaller than any true piroplasm, 2–5 % of the corpuscles contain two to four parasites, and 1–5 % containing dividing, or "cross-forms." The accompanying diagram represents our conclusions as to the usual mode of multiplication of *N. equi* in the circulating blood; we studied the parasite in the living condition as well as in stained films.

*Nuttalliosis* resembles piroplasmosis in its symptomatology and pathology. I have a "salted" horse whose blood was virulent when last tested, a period of three years having elapsed since it was first infected. In Africa, as first shown by Theiler, the parasite is transmitted by *Rhipicephalus evertsi.* This tick, when fed upon infected horses in its larval and nymphal stages, is infective when adult. We do not know what species of tick serves to carry the parasites in other parts of the globe.

### *East Coast Fever of Cattle.*

This devastating disease, which appears to be confined to Africa, is ushered in by the onset of fever following upon an incubation period of 10–20 days after the cattle have been attacked by the ticks which convey the parasite. The fever continues usually for about 12 days, death taking place 18–34 days after the ticks have attacked the host. The characteristic symptoms of redwater, haemoglobinuria, icterus and anaemia, are absent in East Coast fever. The mortality may amount to 80–90 %. Cattle which are immune to redwater are susceptible to East Coast fever. In marked contrast to what is observed in redwater, we find in East Coast fever, as the disease progresses, that there is no appreciable decrease in the number of red blood corpuscles present in the peripheral circulation.

The cause of the disease, *Theileria parva*, Fig. 11, differs in important respects from the parasites I have previously mentioned, and the disease cannot be communicated by the inoculation of blood containing the parasite even when large quantities—up to 7 litres—are injected.

*Theileria parva* is conveyed by *Rhipicephalus appendiculatus, R. simus, R. evertsi, R. nitens* and *R. capensis,* the first-named species of tick being the usual carrier. The parasites are not hereditarily transmitted in *Rhipicephalus,* but when taken up by the carrier at one stage of its

development the tick is infective in the succeeding stage. When *R. appendiculatus*[1], for instance, sucks *Theileria*-infected blood as a larva the tick is infective as a nymph, or, having sucked infected blood as a nymph, it is infective as an adult. Whereas in redwater, the parasites persist in the blood for years after recovery, and "salted" animals are capable of infecting ticks, the contrary holds for *T. parva*, *i.e.* when animals recover from East Coast fever they are incapable of infecting the ticks.

The parasites are at first present in small numbers, but 5–6 % of the corpuscles being invaded. The number of parasites, as a rule, steadily increases until death, when 60–75 % of the corpuscles in the peripheral blood are found to be infected. We have never observed multiplication of the living parasites in corpuscles, but we have seen them in a few rare instances escape from the corpuscles into the plasma. The parasites

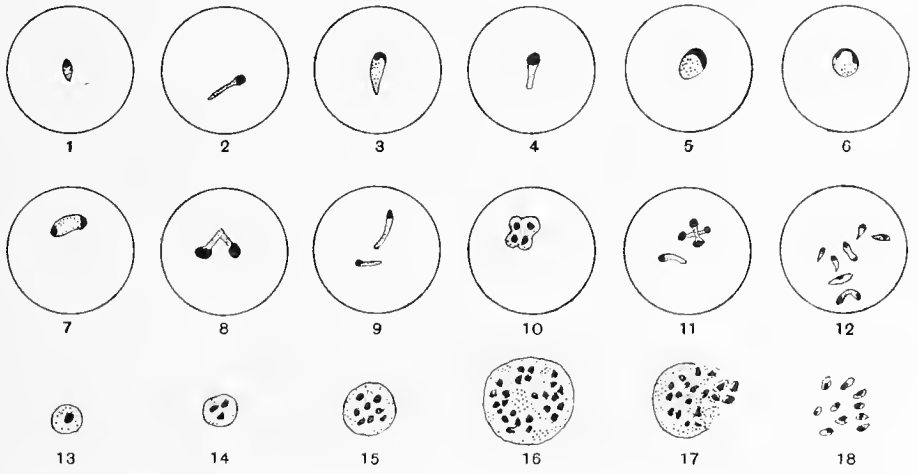


Fig. 11. *Theileria parva*: (1–12) stained intracorpuscular parasites; the figures arranged arbitrarily in a manner which would appear to indicate that they may divide into two or four parasites at a time, although division has not been observed to take place in living parasites; (13–18) Koch's bodies, a series of stained parasites from a spleen smear, likewise arranged arbitrarily in sequence to indicate their probable mode of development from unicellular bodies; (17 and 18) represent the breaking-up of the Koch's body and the liberation of its component elements. The black denotes chromatin, the stippling denotes the blue-staining protoplasm (Giemsa stain). (Original, and from Nuttall and Fantham, 1910.)

move about actively within the corpuscles. Whilst the parasite is very pleomorphic, the commonest forms seen in stained preparations are ovoid or rounded and comma-shaped or clubbed. A proportion of the parasites are bacilliform. The appearance of the chromatin in some parasites

[1] See *Parasitology*, VI. pp. 111–117, 195–203.

suggests that multiplication may occur within the infected corpuscles, some of which contain up to eight distinct parasites. If, however, multiplication occurs within the corpuscles it must take place very slowly or we should have observed it in the living parasite.

The results of our investigations forced me to the conclusion that the corpuscles merely serve as vehicles for the parasites wherein they are housed and maintained until they reach their destination within the tick, which serves as their vector. The seat of multiplication and invasion of corpuscles appeared to me to lie in the internal organs, a view which subsequently received support from the investigations of Meyer, who communicated the disease by the intraperitoneal transplantation of large pieces of infected spleen.

Another striking feature connected with these parasites is the occurrence of "Koch's blue bodies" in the internal organs and occasionally in the peripheral blood. These bodies (Fig. 11, 13–18) were first observed by Koch and to-day are considered to be of prime diagnostic importance. They are termed "blue" because of their appearance in blood-films stained by any of the modifications of the Romanowsky method. When examined stained they usually appear rounded, the blue-staining protoplasm containing discrete chromatin masses in varying numbers; at times they are seen to be breaking up, each mass of chromatin being accompanied or surrounded by blue-staining protoplasm. These bodies, which were regarded as developmental forms by Koch, have been made the subject of detailed study by Gonder, who states that he has seen them break up into their elements and scatter. They are encountered in the internal organs (lymphatic glands, spleen, etc.) before they appear in the corpuscles of the peripheral circulation. In some cases of East Coast fever the parasites do not appear in the corpuscles, whilst Koch's bodies are found only in the internal organs. The bodies are found either free in the plasma or in cells—chiefly in lymphocytes—exceptionally in leucocytes. According to Gonder, these bodies (which he terms "agamonts") undergo multiplication by schizogony, and I am inclined to agree with him. According to this view the parasites are first uninuclear, they grow, and the nucleus divides repeatedly until many small nuclei are formed, after which the minute parasites separate and scatter. It is presumably these small parasites which invade the corpuscles. The rest of the cycle of development, as outlined by Gonder, is so purely conjectural that we can afford to ignore it for the present. It is reasonable to assume, from what I have previously stated, that some of the intracorpuscular parasites represent sexual forms destined

to undergo further development in ticks. That such a development occurs still remains to be determined although Gonder believes that he has partially traced it.

*Remarks upon some other Parasites which appear related to the foregoing.*

In the foregoing instances we have dealt mainly with three distinct forms of intracorpuscular parasites whose mode of transmission, through the agency of ticks, has been demonstrated repeatedly by experiments conducted either upon cattle, horses, sheep or dogs. All of these parasites have until recently been grouped in the one genus together with certain others to which I shall refer more briefly. It has long been evident to me that the genus *Piroplasma*, or *Babesia*, was being regarded as a "hold-all" for parasites of very varied character, and it appears necessary to separate them more clearly. Dismissing, then, the three genera I have dealt with, viz. *Piroplasma*, *Nuttallia* and *Theileria*, we may consider the following:

"*Piroplasma mutans*" Theiler, 1907.

This parasite occurs in cattle in South Africa and Madagascar; it is very minute, resembles *Theileria parva* microscopically but for the absence of Koch's blue bodies, and is likewise transmitted by ticks. Contrary to *Theileria*, the parasite is, however, readily transmitted from animal to animal by blood inoculation, and is practically harmless in respect to pathogenic effects. The parasite is conveyed by *Rhipicephalus simus*, *R. evertsi*, and, rarely, by *R. appendiculatus*. When animals are inoculated, a long period of incubation (60–115 days) elapses before the parasites appear in the blood. These data regarding *P. mutans* are based upon the confused accounts given by Theiler and a few statements made by Gonder. I have only studied the parasite in a few stained blood-films. It is clear that the parasite requires to be adequately studied before its position can be determined.

I would refer briefly, moreover, to the following parasites which have been observed in different animals but regarding which we require more information:

*Rossiella rossi* (Nuttall, 1910), found in the jackal (*Canis adustus*) in British East Africa. The parasite was referred provisionally by me to the genus *Piroplasma*, but subsequently to a separate genus (1912).

The parasites (Fig. 12) are of large size, usually rounded, occurring singly, in pairs, and occasionally in fours. The nucleus of the parasite is very large and rounded when at rest. Multiplication occurs by direct division of the nuclei, two daughter parasites resulting which may in turn subdivide in a similar manner.

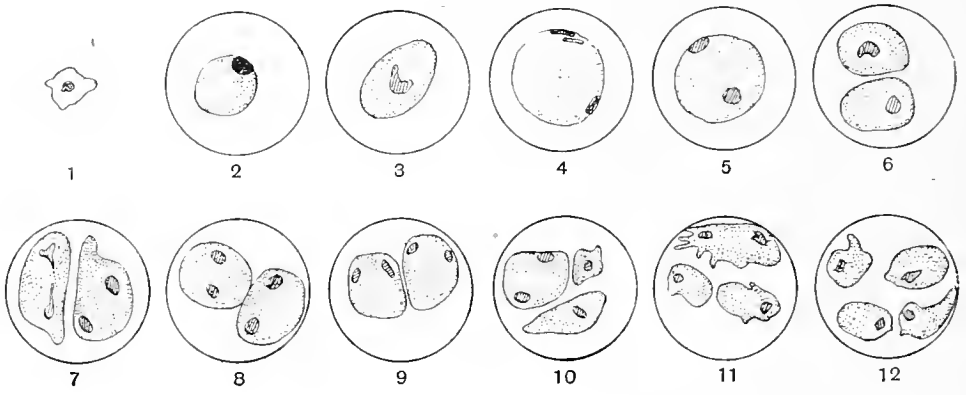


Fig. 12. *Rossiella rossi* (Nuttall, 1910): (1) single free parasite; (2-3) single uninucleate intracorpuscular parasites; (4–12) progressive stages of division into four parasites.

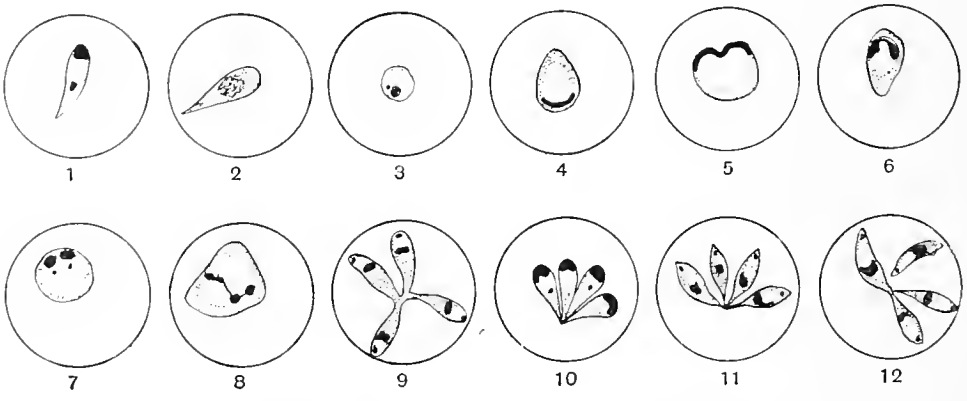


Fig. 13. *Nicollia quadrigemina* (Nicolle, 1907): different stages drawn from a blood-film kindly lent by Prof. C. Nicolle. (Original, G. H. F. N. del.)

*Nicollia quadrigemina* (Nicolle, 1907), found in the gondi (*Ctenodactylus gundi*)—a rodent—in Tunisia by Nicolle, and referred by him to the genus *Piroplasma*; but it differs markedly from the members of this or any other genus and a new genus was therefore founded for it by me in 1908. The parasites (Fig. 13) are oval or piriform, are commonly grouped in fours, often in a fan-like manner, and show distinct binuclearity.

I am not clear as to the position of a considerable number of parasites in different animals and which have been referred to the genera *Piroplasma*, *Theileria*, *Achromaticus* and *Microsoma* by various authors. These parasites require further study before they can be definitely classed.

*Treatment and Prevention.*

In concluding, I must mention the subjects of treatment and prevention. The only drug hitherto discovered which exerts an influence on some of the parasites I have described is Trypanblue[1] a dye which is administered intravenously in preference to subcutaneously in 1–1·5 %

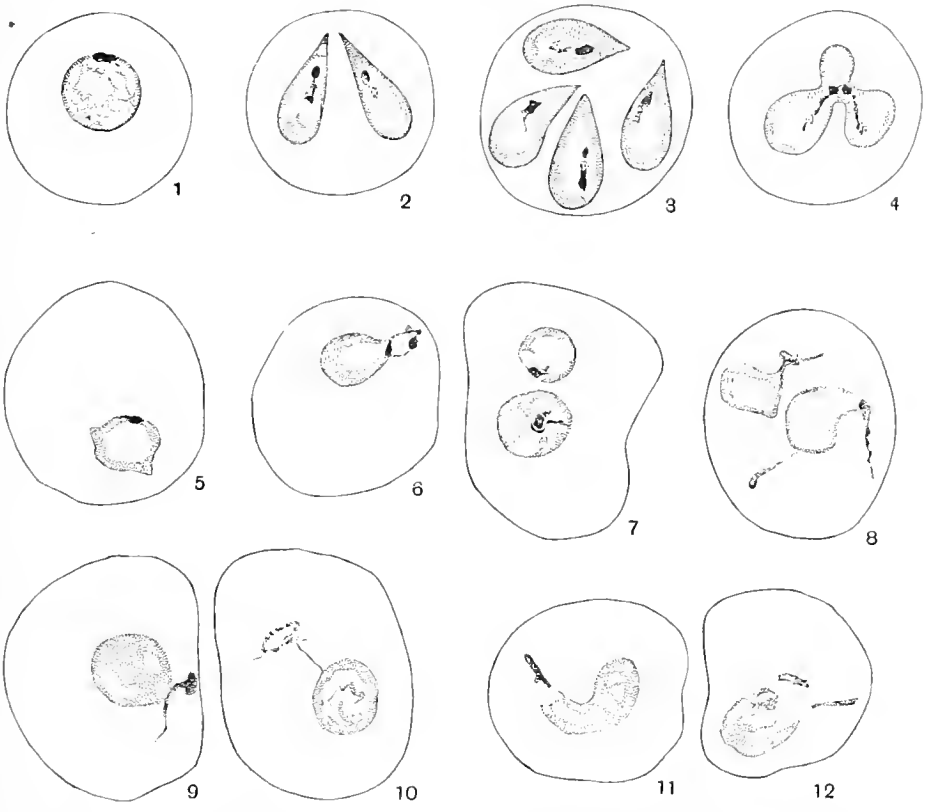


Fig. 14. Showing the effect of Trypanblue upon *Piroplasma canis* in the peripheral blood of a dog: (1–4) normal types of parasites; (5–12) parasites from the same dog, showing progressive degrees of degeneration in blood removed from the dog's ear-vein six hours after the injection of the drug. (Nuttall, 1910.)

aqueous solution. A dose of 5–10 c.c. is curative for dogs suffering from *Piroplasma canis* infection, and the drug is being used to-day in many parts of Africa where it was previously impossible to keep dogs.

[1] Nuttall and Hadwen (1909).

Trypanblue exerts the same effect on cattle and horses suffering from *P. bovis* and *P. caballi* infection; it has proved of value in practice in the treatment of horses and cattle, the dose being 100–150 c.c. of the solution.

Trypanblue has no effect upon the parasites of East Coast fever, and I have no satisfactory records of its having been tried upon the other parasites I have described.

Under the influence of trypanblue (Fig. 14) *Piroplasma* rapidly degenerates and the parasites can no longer be discovered microscopically in the blood. The fever and haemoglobinuria cease, and the animals recover from the other clinical manifestations of piroplasmosis. The animals continue, however, to harbour the parasites in their blood for years, as can be shown by inoculation into clean animals—in other words, the animals recover in the vast majority of cases from the acute disease, and, especially in dogs, are "salted" like the animals that recover in nature. Whereas untreated dogs usually die, the treated animals usually recover.

I may note that the inoculation of cattle is commonly employed with the object of obtaining "salted" animals which are, naturally, more valuable than the "unsalted," in that they resist reinfection when exposed in endemic areas.

The efforts at prevention have been directed against the ticks which transmit the parasites and their transportation from place to place by their hosts. They consist in the use of dips, most of which contain arsenic, in which the animals, especially cattle, are immersed at intervals of a few days or a week or more. Where there are no forests to be endangered, the burning of the dry surface vegetation has been found of some use in destroying the ticks which infest the pasture lands, and, finally, the removal of cattle for a period from infested pastures leading to the starving out of the ticks (especially *Boophilus*) has been found of use.

The preventive measures have to be based upon a knowledge of the life-histories of the ticks which transmit the parasites, and where they have been feasible and have been carried out intelligently they have been of great benefit; large tracts of country in the United States, Australia and Africa having been rendered almost tick-free by these measures.

# CONDITIONS INFLUENCING THE TRANSMISSION OF EAST COAST FEVER[1].

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(*From the Quick Laboratory, University of Cambridge.*)

In spite of numerous researches the life-cycle of *Theileria parva*, the parasite of East Coast Fever, has not yet been elucidated. Especially is this the case with regard to the life-cycle of the parasite within the invertebrate host, *Rhipicephalus appendiculatus*, for the only account hitherto published, namely that of Gonder (1911), is very incomplete and has not been confirmed. Accordingly, we have performed various experiments and examined the organs of numerous ticks in the attempt to throw some light on the subject, and, if possible, confirm Gonder's account of the life-cycle of *Theileria parva* within the invertebrate host. Although we have examined both smears and sections made from all parts of the bodies of more than twenty infected ticks, we have not been able to detect any appearances which could not be found in normal ticks, when the tissues of both the infected and clean ticks were searched with equal diligence. Considering that practically 100 % of the ticks which imbibe the parasites are provedly infective, the number we examined should have sufficed for their detection. Our attempts to

[1] The expenses in connection with this investigation are being defrayed by the aid of a grant from the Rockefeller Institute for Medical Research.

trace the morphological changes undergone by the parasite having thus been negative, we performed some experiments on the effect of various conditions on the infectivity of the ticks, in order to understand more fully the biology of the parasite in the tick. Our results, although still incomplete, are of interest, as they show the nature of some of the conditions affecting the infectivity of *R. appendiculatus* that have fed on cattle infected with East Coast Fever.

Theiler (1904), and Lounsbury (1906), have shown that *Theileria parva* may be transmitted by *Rhipicephalus appendiculatus, simus, evertsi, nitens*, and *capensis*, respectively. In every case the infection is conveyed by the tick only in the stage following engorgement on an infected animal.

Accordingly, a tick having ingested infected blood as a larva will only be infective as a nymph, or, having sucked infected blood as a nymph, it will be infective as an adult. A tick which is infective as a nymph necessarily will be non-infective as an adult, the infection only persisting from one stage to the next. It has been shown that infected nymphs lose their infection and become "clean" by engorging themselves, not only on cattle, but also on rabbits and sheep, animals which are not susceptible to East Coast Fever (Gonder, 1911).

## I. *Experiments on the effect of incomplete feeding on the infectivity of* R. appendiculatus *infected with* Theileria parva.

Our attention was first directed to this subject by Colonel H. Watkins-Pitchford, F.R.C.V.S., who informed us that, in Natal, he had been able to infect cattle with East Coast Fever by the bites of ticks that had previously fed for two or three days on a normal animal. His experiments, which have never been published, showed that ticks do not necessarily become "clean" as a result of biting a vertebrate host, and the importance of this discovery is such that we decided to repeat and also, if possible, extend Watkins-Pitchford's observations in Natal.

Our experiments entirely confirm his discovery and, moreover, demonstrate that an infected tick does not become infective until it has fed on a bovine for at least two days.

The experiments were as follows:

Numerous larvae of *R. appendiculatus* were allowed to gorge themselves on an infected calf shortly before the animal died of East Coast Fever. The gorged larvae dropped off the calf February 6 and 7, and were kept at 30° C. until the nymphs started to emerge six or seven

days later. From February 20 onwards the nymphs were kept at a temperature of about 20° C. These infected nymphs were used in all the following experiments, with the exception of A, B, and C.

*Exp.* 1. (*Control.*)

In order to test their infectivity, on March 4 fifty of these nymphs were placed on a large calf, which was kept in a heated stall at a temperature of about 16° C. Ten days later the calf showed a rise in temperature and after another five days parasites were first seen in the peripheral blood. The animal died of East Coast Fever on March 31, twenty-seven days after the infected nymphs had been placed on it.

*Exp.* 2. *Demonstrating the infectivity of ticks after preliminary feeding on a rabbit for three days.*

On March 4, numerous nymphs were placed on a rabbit which was kept at a temperature of about 20° C. Three days later, 140 of these partially engorged ticks were pulled off the rabbit and placed on a calf. The ticks at once attached themselves to the second host. Ten days later the calf showed a rise in temperature, parasites being first seen four to five days later. The calf died of East Coast Fever on April 3, twenty-seven days after the partially fed nymphs had been placed on it.

It is noteworthy that, in this case, the incubation period is identical with that of the control animal (Exp. 1), the preliminary feeding on a rabbit for three days apparently having had no effect either on the infectivity of the ticks or on the incubation period of the disease.

*Exp.* 3. *Demonstrating that ticks are non-infective during the first two days after their becoming attached to a host.*

On April 9, fifteen infected nymphs, kept overnight at 30° C., were placed on the ear of a calf. Two days later the partially fed ticks were all removed from the animal (see below, Exp. 5). The calf was under observation for five weeks and daily records of its temperature were kept, but the animal never showed fever or any other signs of infection.

In order to determine whether this calf was immune to East Coast Fever, on May 14 forty infected nymphs (belonging to the same lot of

ticks) were placed on the animal and allowed to gorge themselves. Thirteen days later the calf showed a rise in temperature, parasites appeared in its blood after four days, and on June 11 it died of East Coast Fever.

From the above described experiment it seems that *R. appendiculatus*, infected with *Theileria parva*, do not become infective until they have fed on a bovine for more than two days.

The result of Exp. 3 suggests at least two possibilities with regard to the conditions necessary before an infected tick becomes infective. It might be supposed that the parasites within the infected ticks require a certain degree of warmth in order to complete their development and be capable of infecting cattle. The body-warmth of the host might afford the necessary degree of heat, and therefore the following experiment was performed in order to test this hypothesis:—

*Exp.* 4. *Demonstrating that heating unfed infected ticks to* 37° *C. for three days does not render them infective during the first two days after they become attached to the host.*

Nineteen infected nymphs were placed for three days in an incubator at 37° C., prior to being placed on a calf. After having fed on the calf for two days, the partially engorged ticks were removed (see below, Exp. 5) and the animal kept under observation for two months, during which period it never showed any symptoms of being affected.

This experiment shows the incorrectness of the above-mentioned hypothesis. If warmth was the only necessary factor, the ticks, having been heated to body temperature for three days and then placed on the calf for another two days, should have been infective, as, ordinarily, a nymph only requires five days for its complete engorgement. The only alternative hypothesis is that the parasites contained within the unfed and infected tick are unable to complete their development within the invertebrate host until the latter has begun to ingest blood. After the tick has commenced feeding, the influx of blood starts some development resulting in the infected tick becoming infective only after an incubation period of at least two days. In this connection, the result of the following experiment is of some interest:—

*Exp. 5. Demonstrating that preliminary feeding of infected ticks for two days, followed by starvation for seventeen days, renders them non-infective.*

The partially engorged ticks that had been removed from the calves in the two previous experiments (3 and 4) were kept at room temperature (about 20° C.), those from Exp. 3 for eighteen days and those from Exp. 4 for fifteen days, after being taken from the calves. Then both lots were kept overnight at 37° C. and placed on a calf. Only six nymphs were left alive, and these were allowed to engorge on the calf. This animal was kept under observation for four weeks during which period it never showed any rise in temperature. It was then proved to be susceptible to East Coast Fever by allowing 40 infected nymphs to feed upon it. After a normal incubation period the animal fell ill, and died from the disease twenty-seven days after the ticks had been applied.

Although the number of ticks (six) used in this experiment is rather small, Theiler, Gonder and other observers, are all agreed that practically 100 % of *R. appendiculatus* become infected when fed on an infected animal. Therefore, it seems that ticks lose their infection within seventeen days of ingesting blood, and we are led to assume that the developmental processes started by the ingestion of blood into the tick, continue, even if the latter is removed from its host prior to being fully engorged. Apparently, however, when once started, this development of the parasite results in its own destruction, unless it is able to enter the blood of a bovine animal within a period certainly less than seventeen days.

II. *Inoculation experiments with emulsions of infected ticks.*

Since our experiments showed that ticks are non-infective until after they have fed for more than two days, it appeared reasonable to suppose that the reason why all attempts to infect cattle by the inoculation of ticks had hitherto failed was owing to the parasites having been inoculated when they were not at a suitable stage of development. Therefore, the following inoculation experiments were performed:—

On May 14, forty infected nymphs were placed on a calf. Five days later thirty-seven ticks were removed from the animal and divided into two lots. The total contents of nineteen of these gorged ticks, emulsified in 0·6 % saline, were injected into one calf, whilst the salivary glands of

eighteen ticks, similarly treated, were injected into another calf. The animal on which the ticks had engorged became infected after an incubation period of thirteen days and died of East Coast Fever fifteen days later. The two calves that had been inoculated with tick contents were kept under observation for three months, but both remained healthy.

We cannot explain the negative results of these experiments, and are continuing our investigations on the subject.

### III. *Experiments on the effect of cold on the infectivity of* R. appendiculatus *infected with* Theileria parva.

In our efforts to determine what factors influence the development of *Theileria parva* in the tick, our attention was at once directed towards the effects of low temperature. It is well known that *Stegomyia fasciata* cannot transmit Yellow Fever if the temperature falls below about 24° C., and it might be assumed that temperature exerts a similar effect upon the transmission of East Coast Fever by *R. appendiculatus*.

It may be noted that Theiler (1909) exposed ticks infected with East Coast Fever to varying degrees of cold for different periods. His remarks were as follows (*loc. cit.* p. 59):—

"(1) a temperature of 0° C. retards the hatching of brown ticks into adults, (2) a temperature of 0° C. does not interfere with the development of the parasite within the engorged nymphae, (3) a temperature of 0° C. does not kill the virus contained in engorged nymphae of the brown tick. In no instance was any difference noted in the virulency of the disease; the only point of interest was that the ticks kept at a low temperature moulted at a later date than the controls, but when the former were placed on susceptible cattle these animals promptly contracted the disease and died." Theiler does not give any particulars of his experiments, and we are unable to explain the discrepancy between his results and those obtained in our experiments.

On October 30, a number of larvae of *R. appendiculatus* were placed on a calf heavily infected with East Coast Fever. The gorged larvae dropped off three to five days later and the calf died of the disease as the last larvae dropped off. The gorged larvae were kept in a greenhouse at a temperature of about 25° C., and the infected nymphs began to emerge about a week later. From December 13 onwards the nymphs were kept at a temperature of about 19° C. These ticks were used in the following experiments (A, B, C):—

*Exp. A. (Control.)*

On January 18, thirty of these nymphs were warmed for one night to a temperature of 37°C., and the following day placed on a calf and allowed to gorge themselves. This animal showed parasites in its blood thirteen days later, and died of East Coast Fever twenty-three days after the nymphs had been placed on it.

*Exp. B. Demonstrating the non-infectivity of infective ticks maintained at about 10° C. for three weeks.*

The nymphs, having thus been shown to be infected, were placed in the cellar on February 25 at a temperature of about 10° C. After having been kept at this temperature for twenty-one days (until March 18) 105 of these nymphs were placed, without any previous warning, on a calf which was kept in the cold stall at ordinary out-door temperatures (about 8° C.).

The gorged nymphs dropped off five to eighteen days later, and the calf was kept under observation for a month (until April 16) during which period it never showed any rise in temperature nor any other symptoms of East Coast Fever.

On April 16, thirty infected nymphs that had been kept at a temperature of 22° C. were placed on this calf, which was still kept in the cold stall. After an incubation period of eleven days the calf showed a rise in temperature and died of East Coast Fever twenty-one days later, in this case the disease having a somewhat prolonged course.

This experiment clearly shows that nymphs of *R. appendiculatus* infected with *Theileria parva*, after being exposed for three weeks to a temperature of about 10° C., lose their infectivity and become innocuous to cattle.

*Exp. C. Demonstrating the effect of warmth in restoring the infectivity of ticks previously non-infective from exposure to cold.*

Having found that a low temperature causes a tick to become non-infective, it remained to be seen whether the infectivity could be restored by subsequent warming. For this purpose some of the nymphs from Exp. B, were again kept at a temperature of about 20° C. for a period of four weeks, and, finally, for two days at 30° C. Forty of these nymphs were then allowed to gorge themselves on a calf. Thirteen

days after this animal showed its first rise in temperature and fifteen days later died of East Coast Fever.

It seems, therefore, that the effect of cooling ticks infected with *Theileria parva* is to cause the parasite to become incapable of completing its development when the tick feeds. The parasite is not destroyed by the low temperature, as evidenced by the ticks again becoming infective on being re-heated to 20° C. This non-infectivity of *R. appendiculatus* at low temperatures resembles closely that of *Argas persicus* infected with *Spirochaeta gallinarum*, for, as shown by Borrel and Marchoux (1905), when infected *Argas* are kept at low temperatures they cease to be infective, but on again heating the ticks to a temperature of 35° C. they recover their infectivity.

## Summary.

The foregoing experiments on the transmission of East Coast Fever by *Rhipicephalus appendiculatus* appear to permit of the following conclusions:

1. Infected ticks do not produce infection during the first two days when feeding on cattle.
2. Infected ticks are still infective after feeding upon a rabbit for three days.
3. Heating infected ticks to 37° C. for three days does not render them infective during the first two days after they become attached to the host.
4. The partial feeding of infected ticks for two days, followed by starvation for seventeen days, renders them non-infective.
5. Inoculations of emulsions of infective ticks collected from cattle on the fifth day of engorgement failed to produce infection.
6. Infective ticks are rendered non-infective by exposure to a temperature of about 10° C. for three weeks.
7. Their infectivity may be restored by subsequently warming them.

By way of a working hypothesis, we assume that the final development of the parasite in the tick, resulting in the latter becoming infective, only commences after the tick has begun to ingest blood.

Although our investigations are still in progress, we consider them of sufficient interest to warrant their publication at this stage.

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## *Protocols of experiments.*

*Calf* 1.

| | Day | Temp. °F. | |
|---|---|---|---|
| 4. iii. 13 | 1 | — | Placed fifteen infected nymphs on the calf. |
| | 2–4 | Normal | |
| | 5 | ,, | One gorged nymph dropped off. |
| | 6 | ,, | Sixteen gorged nymphs dropped off. |
| | 7 | ,, | Eight ,, ,, ,, |
| | 8–11 | ,, | |
| | 12 | 103·6 | |
| | 13 | 103·4 | |
| | 14 | 105 | |
| | 15 | 106·2 | |
| | 16 | 105·8 | Few parasites found in blood. |
| | 17 | 107·4 | |
| | 18–30 | Temperature ranging from 104·4° to 107° F. | |
| 31. iii. 13 | 28 | — | Calf died of East Coast Fever. |

*Calf* 2.

| | | | |
|---|---|---|---|
| 7. iii. 13 | 1 | — | Put on about 140 nymphs that had previously fed on a rabbit since 4. iii. 1913 (3 days). |
| | 2–3 | Normal | |
| | 4–8 | ,, | Gorged nymphs dropped off. |
| | 9–10 | ,, | |
| | 11 | 104·6 | |
| | 12 | 105·5 | |
| | 13–27 | Temperature ranging from 104° to 106·8° F. | |
| 3. iv. 13 | 28 | — | Calf died of East Coast Fever. |

*Calf* 3.

| | Day | Temp. °F. | |
|---|---|---|---|
| 9. IV. 13 | 1 | — | Put on fifteen infected nymphs. |
| | 2 | Normal | |
| | 3 | ,, | Partially fed ticks all removed from the calf. |
| | 4–35 | ,, | |
| 4. V. 13 | 36 | ,, | Put on forty infected nymphs. |
| | 37–39 | ,, | |
| | 40–43 | ,, | Gorged nymphs dropped off. |
| | 44–49 | ,, | |
| | 50 | 105 | |
| | 51 | 107 | |
| | 52 | 104 | |
| | 53 | 104·4 | Few parasites found. |
| 1. VI. 13 | 54 | 105 | |
| | 55 | 105·8 | |
| | 56 | 105·6 | |
| | 57 | 105·2 | |
| | 58 | 105·6 | |
| | 59 | 103·4 | |
| | 60 | 102·1 | |
| | 61 | 102·4 | |
| | 62 | 104·4 | |
| | 63 | 106·4 | |
| 11. VI. 13 | 64 | 105·6 | Calf died of East Coast Fever at 6.35 p.m. |

*Calf* 4.

| | | | |
|---|---|---|---|
| 12. IV. 13 | 1 | — | Put on nineteen infected nymphs previously heated to 37° C. for three days. |
| | 2 | Normal | |
| | 3 | ,, | Nineteen partially gorged nymphs removed. |
| | 4–80 | ,, | |
| | 81 | — | Experiment discontinued. |

*Calf* 5.

| | | | |
|---|---|---|---|
| 29. IV. 13 | 1 | — | Six partially gorged nymphs from Calves 3 and 4 placed on ear of calf. |
| | 2–5 | Normal | |
| | 6–7 | ,, | Six gorged nymphs dropped off. |
| | 8–15 | ,, | |
| | 16 | ,, | Put on forty infected nymphs. |
| | 17–28 | ,, | |
| | 29 | 101·3 | |
| | 30 | 105 | |
| | 31–44 | Temperature ranging from 104° to 107° F. | |
| | 45 | — | Calf died of East Coast Fever. |

*Calf* 6.

| | Day | Temp. °F. | |
|---|---|---|---|
| 19. v. 13 | 1 | — | Calf injected subcutaneously with the total contents of nineteen gorged infected nymphs from Calf 5. |
| | 2–60 | Normal | |
| | 61 | — | Experiment discontinued. |

*Calf* 7.

| | | | |
|---|---|---|---|
| 19. v. 13 | 1 | — | Calf injected subcutaneously with the salivary glands of eighteen gorged nymphs from Calf 5. |
| | 2–60 | Normal | |
| | 61 | — | Experiment discontinued. |

*Calf* 8. (Exp. A.)

| | | | |
|---|---|---|---|
| 18. i. 13 | 1 | — | Put on thirty infected nymphs. |
| | 2–3 | Normal | |
| | 4–8 | ,, | Gorged nymphs dropped off. |
| | 9 | 105·4 | |
| | 10 | 105 | |
| | 11–22 | Temperature ranging from 105° to 107·4° F. | |
| | 23 | 107 | |
| 11. ii. 13 | 24 | 101·6 | Calf died of East Coast Fever in the morning. |

*Calf* 9. (Exp. B.)

| | | | |
|---|---|---|---|
| 18. iii. 13 | 1 | — | Put on 105 infected nymphs that had been previously kept for three weeks at 8–10° C. Calf kept in cold stall at out-door temperature (about 8° C.). |
| | 2–5 | Normal | |
| | 6 | ,, | One gorged nymph dropped off. |
| | 7–10 | ,, | |
| | 11 | ,, | Four gorged nymphs dropped off. |
| | 12–13 | ,, | |
| | 14 | ,, | Eight ,, ,, ,, |
| | 15 | ,, | Five ,, ,, ,, |
| | 16 | ,, | Four ,, ,, ,, |
| | 17–18 | ,, | |
| | 19 | ,, | One gorged nymph dropped off. |
| | 20–30 | ,, | |
| | 30 | ,, | Put on thirty infected nymphs, kept at 22° C., and heated to 37° C. over night, before being placed on calf. |
| | 31–41 | ,, | |
| | 42 | 104·4 | |
| | 43–61 | Temperature ranging from 104° to 105·2° F. | |
| | 62 | — | Calf died of East Coast Fever. |

*Calf* 10. (Exp. C.)

| | Day | Temp. °F. | |
|---|---|---|---|
| 14. v. 13 | 1 | — | Put on forty infected nymphs that had been kept at 8–10° C. for 48 days, then for four weeks at 20° C., and finally for two days at 30° C. |
| | 2–5 | Normal | |
| | 6–9 | ,, | Gorged nymphs dropped off. |
| | 10–12 | ,, | |
| | 13 | 101·2 | |
| | 14 | 104·4 | |
| | 15–17 | Temperature ranging from 104° to 107° F. | |
| | 18 | 104·4 | Parasites first seen. |
| | 19–28 | Temperature ranging from 102·1° to 106·4° F. | |
| | 29 | 105·6 | Calf died of East Coast Fever. |

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